Synthesis of functionalized [2]rotaxanes and their potential use in ion transport and atomic force microscopy

By

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Lectures and Meetings Attended

1. **Organic Research Seminars**, School of Chemistry, University of Edinburgh, Scotland, 2003-2006.
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3. **14th (Scottish) Graduate Symposium on Novel Organic Chemistry,** University of Aberdeen, 04/03
4. **Organon Symposium on Synthetic Chemistry,** University of Glasgow, 09/03.
5. **32nd Scottish Regional Perkin Meeting,** University of Edinburgh, 12/03
6. **UK Macrocycles and Supramolecular Chemistry Meeting,** University of Sheffield, 01/04.
7. **USIC 2004**, Heriot Watt University, Edinburgh, Scotland, 9/04.
8. **RSC Perkin Meeting**, University of St Andrews, Scotland, 11/04.
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List of Abbreviations

Alloc	allyloxy-carbonyl
Boc	*tert*-butyloxy-carbonyl
Cbz	benzyloxy-carbonyl
DMSO	dimethylsulphoxide
DMF	*N,N'*-dimethylformamide
THF	tetrahydrofuran
TFA	trifluoroacetic acid
EtOAc	ethyl acetate
EDCI·HCl	1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride
4-DMAP	4-dimethylaminopyridine
HOBt	N-Hydroxybenzotriazole
Et	Ethyl
Me	Methyl
NMR	Nuclear Magnetic Resonance
ppm	part per million
mins	minutes
δ	chemical shift
E	*trans* isomer
Z	*cis* isomer
m.p.	melting point
TLC	Thin Layer Chromatography
FAB	Fast Atom Bombardment
rt	room temperature
mL	millilitres
μL	microlitres
g	grams
HRMS	High Resolution Mass Spectrometry
Calcd.	calculated
ΔG°	Gibbs energy

General Remarks of Experimental Data

All the melting points (m.p.) were determined using a Electrotermal 9100 melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer and the chemical shifts are reported in part per million (ppm) from low to high field. All the ^{1}H and ^{13}C NMR spectra were recorded at 298K unless otherwise stated. ^{1}H NMR are reported as follows: br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt doublet of triplets, q = quartet, m = multiplet. All compounds have been named using IUPAC/ACD software. Column chromatography was carried out using Kiesegel C60 (Merck) as stationary phase. TLC detection was performed on silica gel plates (0.25 mm thick, 60 F254, Merck, Germany). Mass spectrometry and HRMS analyses were performed by the University of Edinburgh mass spectrometry service using fast atom bombardment (FAB) from *m*-nitrobenzyl alcohol matrix unless otherwise stated. Photoisomerizations were carried out in quartz vessels using a multilamp photoreactor model MLU18 manufactured by Photochemical Reactors Ltd, Reading UK. Reagents and anhydrous solvents used for the reactions were purchased from Aldrich and were, unless otherwise stated, used without further purification. Isophthaloyl dichloride was routinely recrystallized from hexane and *para*-xylylenediamine was distilled under reduced pressure.

Alla mia famiglia

To my family

1 Introduction to molecular machines

Self-organization and self-assembly[1] in functional superstructures are some of the most fascinating properties of biomolecules. The DNA's double helix structure, the blueprint for life itself, is among the abundance of natural occurring examples that take advantage of self-organizing to accomplish a specific function.[2] The design of synthetic systems which are capable of undergoing self-organization, i.e. systems that may spontaneously self-assemble to form well-defined supramolecular architectures, is one of the most recent fields of modern chemistry.

The underlying premise of supramolecular chemistry is: self organization, brought about by use of relatively weak forces such as hydrogen bonding, electrostatic and metal-ion-ligand interactions between the covalent components to form well-ordered larger structures in solution, liquid crystalline phase or in the solid state. In addition, Natures' ability to employ the cumulative effect of several of these forces to selectively bind, or increase the probability of binding, one specific molecule to another, in the presence of many other molecules, is a remarkable feature of many natural systems. The basis for enzyme-substrate association is rooted in this phenomenon, and represents some of the first host-guest interactions to be studied.[3-6] Supramolecular structures resulting from the association of two or more chemical species that are bond reversibly by a series of weak intermolecular forces, rather than by covalent bonds, were discovered and studied more than a decade ago.[1]

1.1 Catenanes and Rotaxanes.

Catenanes[7] (from the Latin word: *catena*, chain) are molecules containing two or more interlocked rings that can be only be separated by breaking a covalent bond (Figure 1 a). Similarly rotaxanes[7] (from the Latin word: *rota*, wheel, and *axis,* axle) are comprised of a dumbbell-shaped "thread" component around which macrocyclic component(s) are encircled. The stoppers are bulky enough to prevent the dethreading of the macrocycle (Figure 1 b).

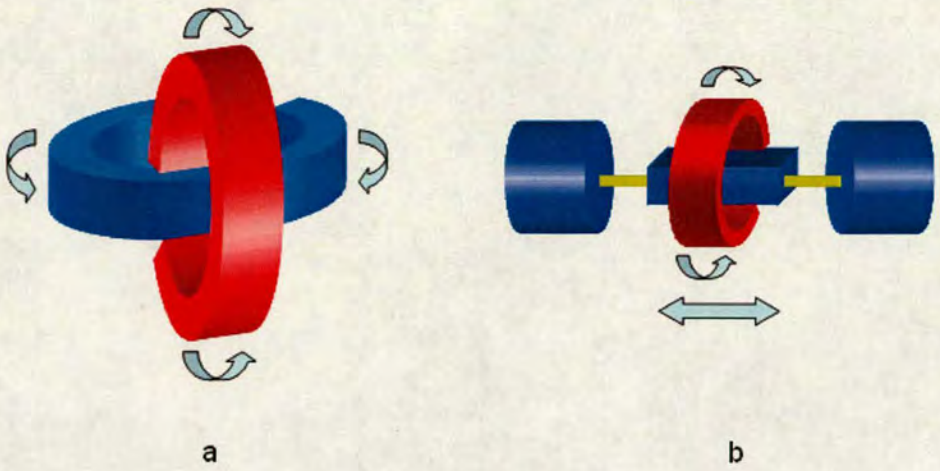


Figure 1. Cartoon representations of a: [2]catenane (a) and a [2]rotaxane (b).

The nomenclature used to describe both rotaxane and catenanes places the number of the components in square brackets before the name of the complex. Therefore, a [2]catenane is comprised of two interlocked macrocycles, and likewise, a [2]rotaxane is comprised of one macrocycle and one thread. The thread and ring (in rotaxanes), or interlocking rings (for catenanes), are not covalently linked to one another, but are only held together mechanically, allowing large amplitude sub-molecular motion of one component relative to the other(s). Pirouetting[7] in a [2]catenane, is the relative rotation of one ring around the other and is the most important large amplitude motion (Figure 1a) whilst in a [2]rotaxane the rotation of the ring around the thread (Figure 1b) is less important. The most important motion in a [2]rotaxane is the translational movement of the ring along the thread, termed "shuttling" (Figure 2b).[7]

1.1.1 Synthesis of interlocked molecules assisted by Supramolecular interactions

The first synthetic routes employed to achieve such molecules were based on statistical approaches that were successful, but inherently extremely low yielding. Wasserman's preparation of interlocking rings was achieved in less than 1% yield.[8] The first synthesis of a [2]rotaxane reported by Harrison and Harrison employed a similar statistical approach with a yield of only 6%.[9] It was not until the realisation that employment of selecting weak interactions would lead to the development of

"directed" synthesis, that both rotaxanes and catenanes and other interlocked molecules could be made in reasonable yields. "Directed" synthesis is mainly (though not only) based on non-covalent interactions identical to those used in molecular recognition (metal-ligand, π-donor π-acceptor, hydrophobic and/or hydrogen bonding),that both rotaxanes and catenanes and other interlocked molecules could be made in reasonable yields.[1][10][11, 12] Two main strategies have been developed for the synthesis of rotaxanes (Figure 2)

- Threading: where the macrocycle is held by non covalent interactions followed by capping of the two bulky stoppers (Figure 2b).[13]
- Clipping: an acyclic unit is enclosed around a thread by templating of the two components. In this case other byproduct species such as catenanes and macrocycles are often formed (Figure 2c).[14]

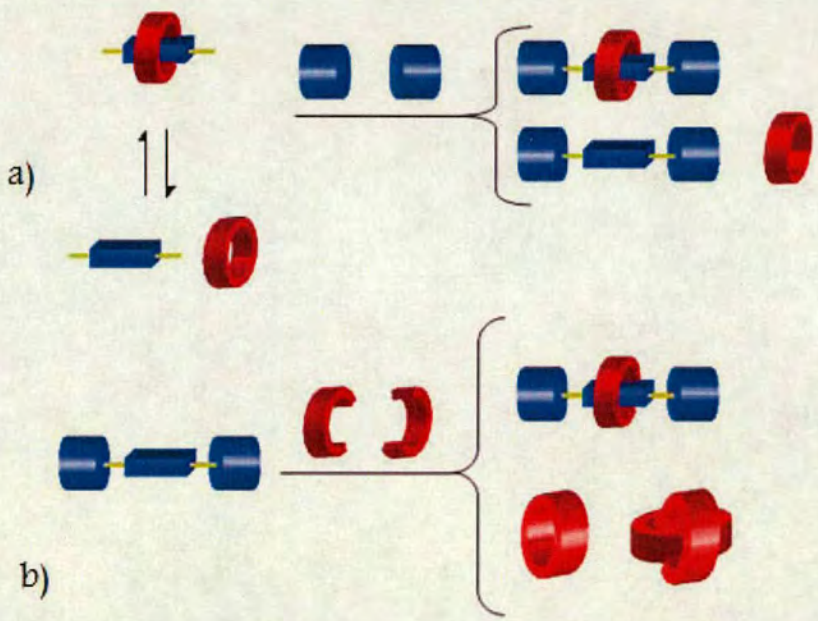
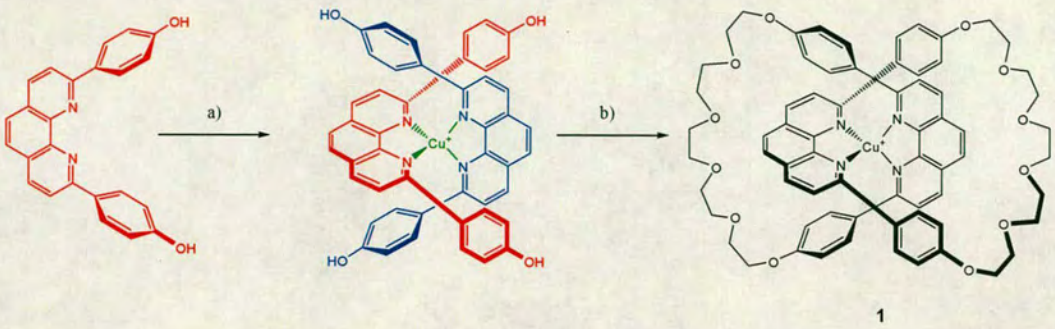


Figure 2. Cartoon representing two different ways of forming rotaxanes. a) Threading and stoppering and b) clipping.

The first supramolecular templated synthesis of an interlocked molecule was reported in 1983 by Sauvage and coworkers (Figure 3)[15] where metal-ligand coordination bonding interactions were exploited. The synthesis involves the use of Cu(I) which adopts a tetrahedral geometry coordinating to the two bidentate

phenanthroline ligands followed by the formation of the two interlocking macrocycles by alkylation of the terminal phenols with iodoethyleneglycol ethers. Catenane **1** was obtained in 27% yield. Subsequently, similar metal-ligand interaction have been used to template the synthesis of rotaxanes exploiting octahedral[16,17] and square planar[18] geometries.



1

Figure 3. First interlocked [2]catenane reported by Sauvage. a) $Cu(NCCH_3)_4^+$; b) Cs_2CO_3, $I\text{-}CH_2(CH_2OCH_2)_4CH_2\text{-}I$.

In 1992 Stoddart and coworkers[19] reported the synthesis of the first [2]rotaxane **2** incorporating a π-electron rich dumbbell-shaped component synthesised by self assembly employing both clipping and threading approaches (Figure 4).

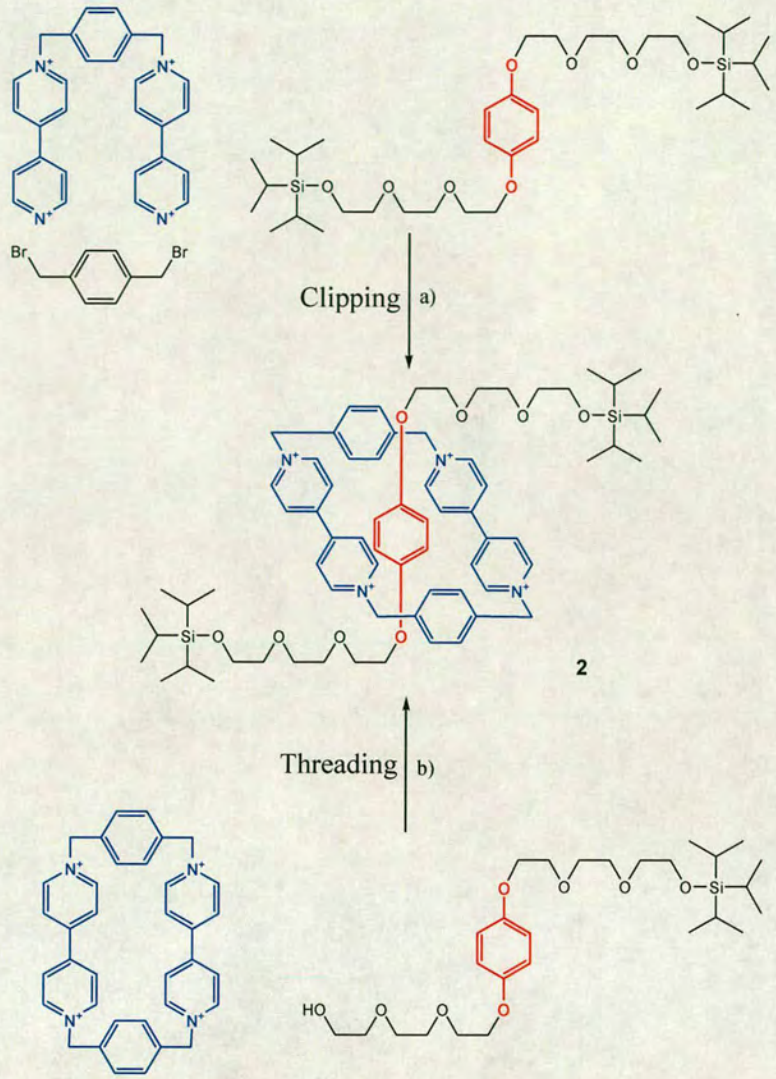
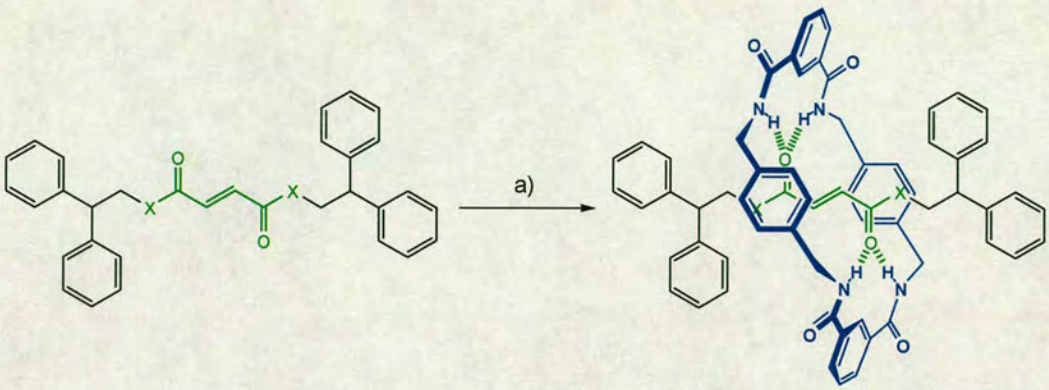


Figure 4. Synthesis of rotaxane **2** reported by Stoddart and coworkers. a) $AgPF_6$, CH_3CN, RT, 7 days, 14%; b) triisopropyl silyltrifluoromethane sulfonate, 2,6-lutidine, CH_3CN, RT, 1h, 22%.

The driving forces for rotaxane formation, in this case, are π- stacking between the π-electron rich hydroquinone unit and the π-electron deficient paraquat ring and hydrogen bonding between the polyether oxygen atoms and the hydrogen atoms on the bipyridinium unit.

Hydrogen bonding interactions were further exploited to form catenanes and rotaxanes by Hunter[20] and Vögtle[21]. In 2001, Leigh and co-workers[22] took this approach to the limit and reported a yield of 97% for the synthesis of the [2]rotaxane

5 templated by hydrogen bonding interactions (Figure 5). The synthesis utilises the preorganisation afforded by the double bond of the fumaramide template **4** which rigidly holds the two carbonyl groups of the thread to form strong bifurcated hydrogen bonds with the tetraamide macrocycle components allowing the formation of the ring.



3 X = O
4 X = NH

5 X = NH, yield = 97%
6 X = O, yield = 3%

Figure 5. Approach to the synthesis of a [2]rotaxane by Leigh. a) Isophthaloyl dichloride, xylylene diamine, Et_3N, $CHCl_3$.

1.2 Rotaxane-based Molecular Motors.

Rotaxanes have fascinated chemists not only for their unique mechanically interlocked structures, but also for their potential in forming the basis of functioning nanoscale devices molecular shuttle and switches.[23] As previously mentioned, the two most important large amplitude movements in [2]rotaxanes are shuttling and pirouetting. Controlling the movement and position of the macrocycle along the thread has over the past thirty years been an interesting challenge.[24] The laws of physics state that, at temperatures above 0 K, molecules move constantly and randomly governed by a phenomenon known as Brownian motion as it was first observed by botanist Robert Brown in 1827 while studying pollen particles suspended in water.

In a [2]rotaxane the macrocycle moves, (via Brownian motion) along the thread and is confined to one dimension and limited by the two bulky stoppers. This motion has become particularly important when studying systems where the thread contains two or more binding sites of different affinities for the macrocycles, wherein controlled movement of the macrocycle from one site to the other along the thread may be realised. The simplest system is one in which a macrocycle shuttles between two isoenergetic stations separated by an energy barrier. Figure 6 shows a cartoon of a [2]rotaxane containing two degenerate and well separated stations (light blue), where a macrocycle (yellow), having equal affinity for either blue station, spends half its time over each, as a consequence of being in isoenergetic states.

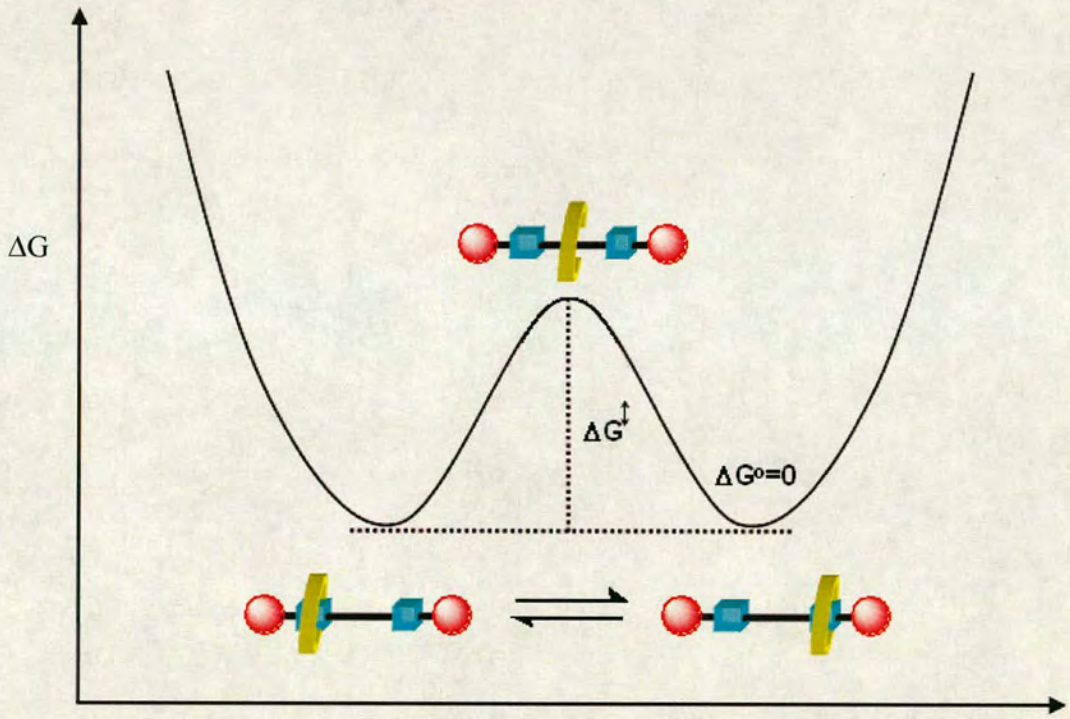


Figure 6. Energy profile of a [2]rotaxane based on two isoenergetic stations.

The first example of a [2]rotaxane featuring degenerate stations came from Stoddart and co-workers in 1991 (Figure 7).[25]

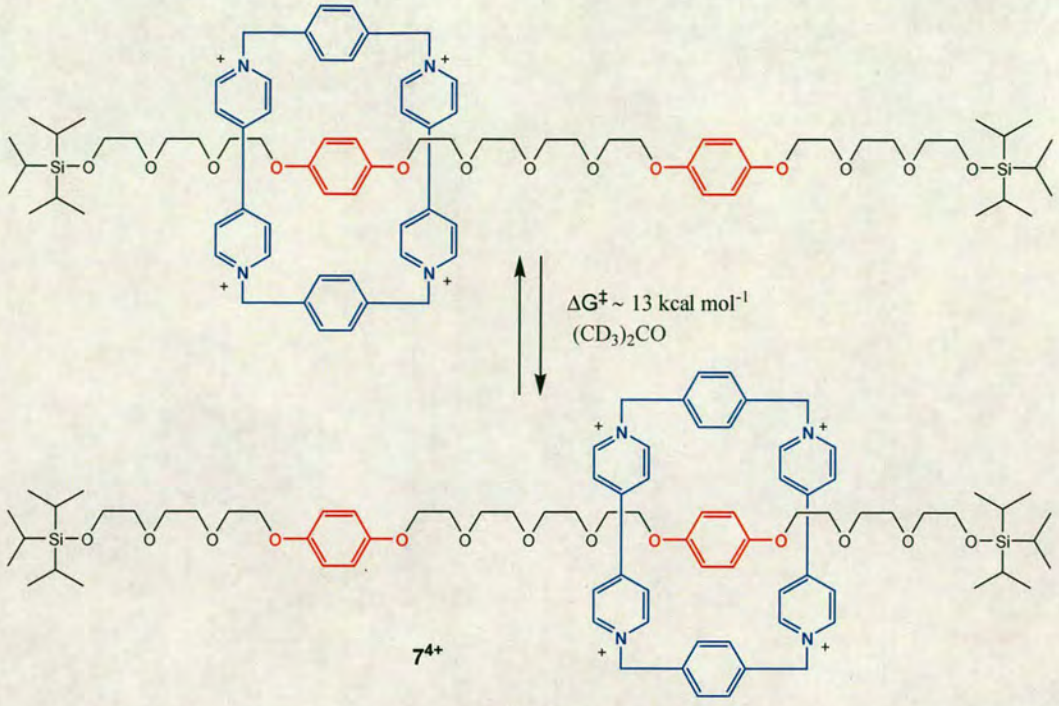


Figure 7. A solvent switchable rotaxane reported by Stoddart and coworkers.

This non stimuli responsive molecular shuttle 7^{4+} is based on a thread containing two electron rich hydroquinol stations (red), a polyether spacer and a tetracationic electron poor cyclophane macrocycle (blue). The affinity of the macrocycle for either of the two stations is due to π-π and charge-transfer interactions. Since the macrocycle has no preference for either of the stations, it spends equal time over each, shuttling back and forth along the thread. With the aid of ^{1}H NMR, it was found to have a rate of 2360 s^{-1} in DMSO at a temperature of 34°C.

Rotaxanes containing two degenerate stations have also been studied in detail by Leigh and co-workers (Figure 8).[26] Threads featuring two identical glycine-glycine peptide stations separated by different spacers were prepared. At room temperature in both rotaxanes **8** and **9** the macrocycle spends equal time over each of the stations in $CHCl_3$ a non hydrogen-bond disrupting solvent. In shuttling from one station to the other the four hydrogen bonds between the macrocycle and the binding site must be broken. Therefore, hydrogen bonding solvents can play an important role since they can form hydrogen bonds with the rotaxane thereby influencing the shuttling process.

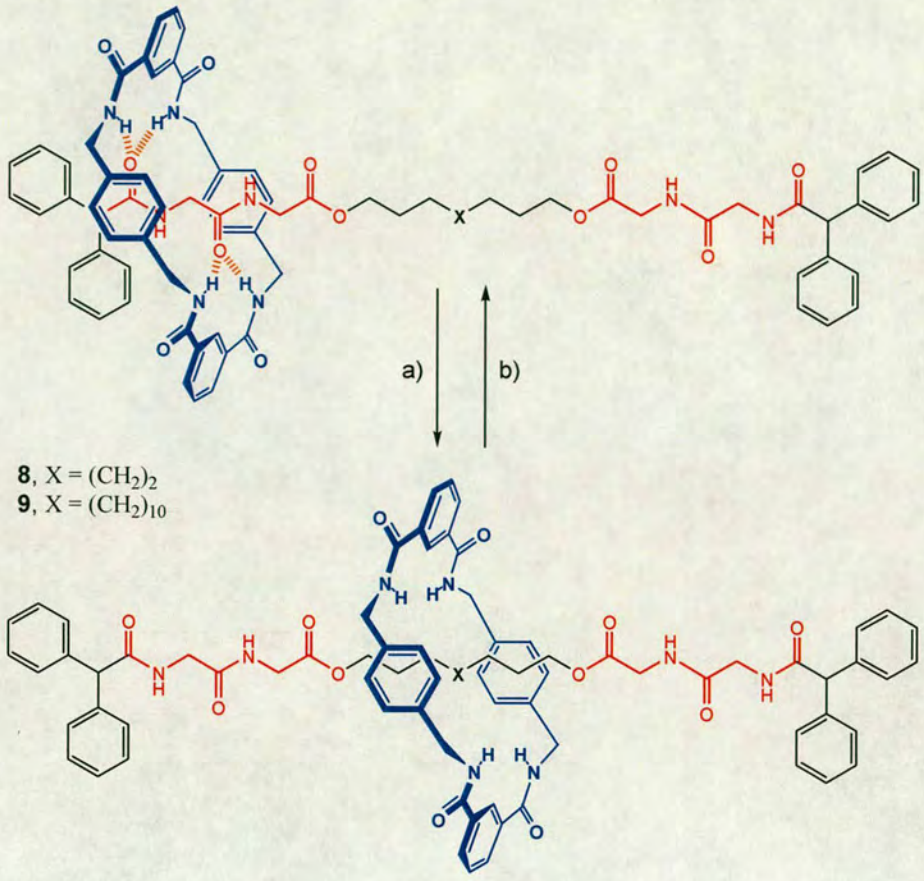


Figure 8. Solvent switchable molecular shuttles **8** and **9** reported by Leigh. a) DMSO-d_6; b) $CDCl_3$.

Given a certain hydrogen bonded assembly, the more polar the solvent is the faster will be the shuttling rate between the two stations in response to competition from the solvent and the situation is significantly reversed when DMSO-d_6 is used instead of $CDCl_3$ as solvent. The ^1H NMR experiments confirm that the macrocycle resides mostly over the alkyl chain spending less time over the two stations. Such systems where the solvent is used to control the shuttling have been named solvent-switchable molecular shuttles.

If the two stations are energetically degenerate, the macrocycle spends equal time on each station. However, if there is a difference in affinity for the macrocycle between the two stations the macrocycle will spend more time over one station. Furthermore

if one of the stations can be modified, for example by an external stimulus, a stimuli responsive molecular shuttle may be designed in which it is possible control of the position of the macrocycle. The important features of a stimuli responsive molecular shuttle are the following (see also Figure 9):

- one of the stations must have two switchable states of differing affinity for the macrocycle (in the example; high affinity: green station, low affinity: light blue station)
- the second station must have an intermediate binding affinity between the two states of the first station (in the example; intermediate affinity: orange station).

The population between the two stations is regulated by a Boltzman distribution; the station with higher affinity will be more populated (i.e. the macrocycle will spend most of the time over the green station). If a stimulus is given that changes the state of the first station (i.e. by switching it from green to blue) the macrocycle will now reside mostly over the second station, which now has the higher affinity in the new system. Application of a second stimulus to switch the green station back to its original state reverses the shuttling process. The stimulus may either destabilize the preferred binding site (stimulus 1), or increase the binding strength of the less populated station (stimulus 2). In both cases the system is put out of equilibrium, and relaxation towards the new energy minimum is achieved by biased Brownian motion.

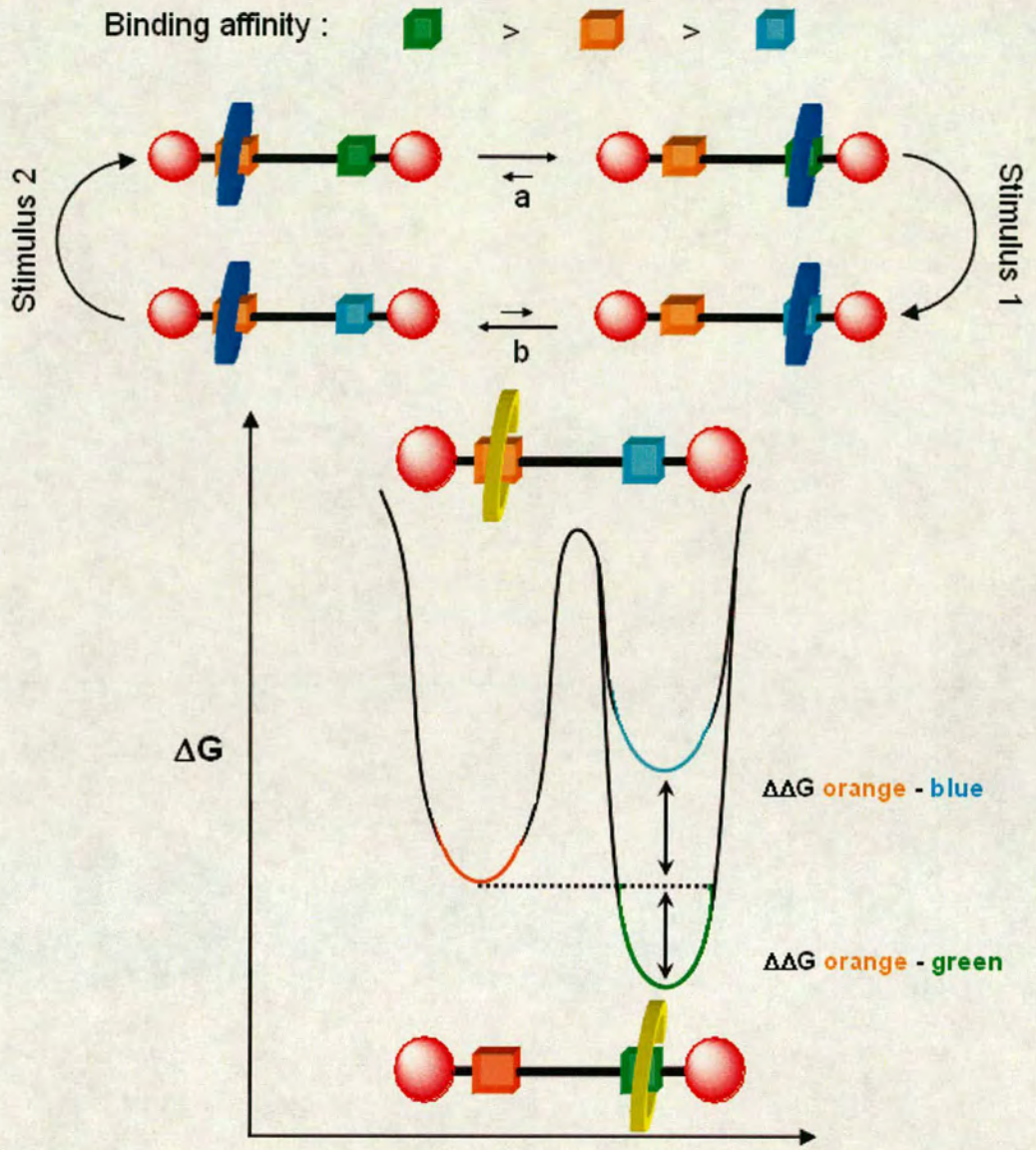


Figure 9. Free energy profiles of a switchable molecular shuttle.

1.2.1 Controlling shuttling by adding or removing protons.

The first example a of stimuli-responsive molecular shuttle was synthesized in 1994 by Stoddart and Kaifer (Figure 10).[27] Rotaxane $\mathbf{10}^{4+}$ is based on the π-electron acceptor cyclophane macrocycle and on a thread containing two different π-electron donor stations. At room temperature the macrocycle occupies both stations to the same degree. However addition of CF_3CO_2D produces the corresponding rotaxane

$\mathbf{10}^{6+}$ where the benzidine station is now protonated. Charge repulsion between the cyclophane macrocycle and the protonated station subsequently results in the macrocycle residing over the neutral biphenol station. Addition of pyridine-d_5 restores the system to its original state.

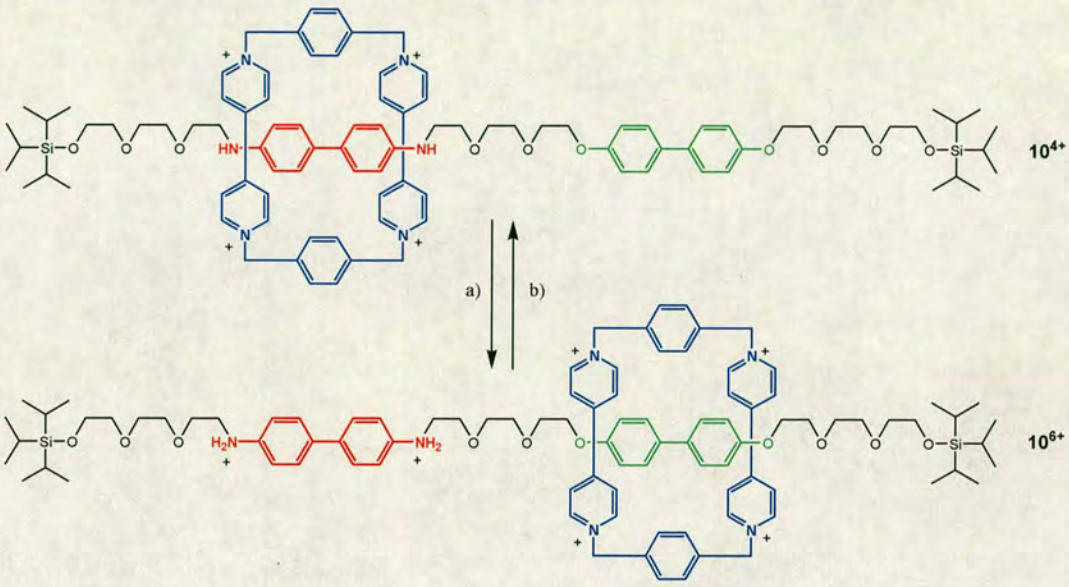


Figure 10. First example illustrating shuttling between two stations in a rotaxane reported by Stoddart.

Stoddart and co-workers have reported another example of switchable molecular shuttles based on the well known interaction of an ammonium group and a crown ether macrocycle. The latter are known to bind ammonium ions by means of hydrogen bonding interaction between the ammonium N-H and the oxygen atoms of the macrocycle. Stoddart's approach to the development of a two station rotaxane was to design a thread based on two different stations with a different affinity for the crown ether; this thread featured a secondary alkylammonium ion and a bipyridinium station (Figure 11).[28, 29] In the protonated system the crown ether binds the alkyl ammonium ion as judged by the shifting of the methylenic protons adjacent to the ammonium ion in the ^{1}H NMR spectra using CD_3COCD_3 as solvent. Treatment of $\mathbf{11}^{3+}$ with a diisopropylethylamine (*i*-Pr_2NEt) resulted in the deprotonation of the ammonium ion reducing its binding affinity with the macrocycle leading to the preferred binding of the bipyridinium station.

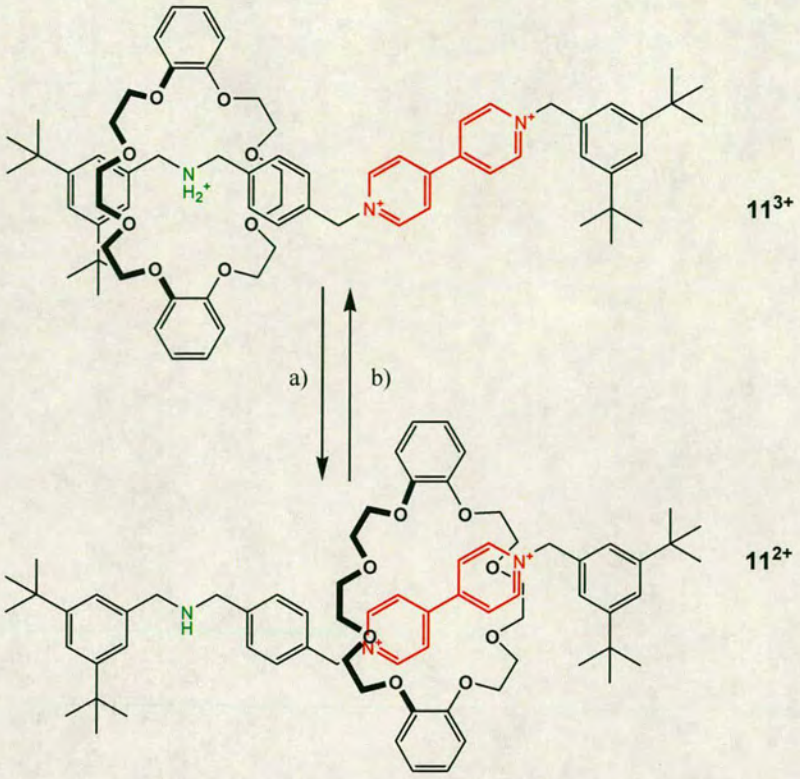


Figure 11. A two station [2]rotaxane with a crown ether based macrocycle a) CF_3COOH b) *i*-Pr_2NEt.

Subsequently, in 2004, Stoddart and co-workers[30] reported a "molecular elevator" **14** based on the above mechanism (Figure 12). The "platform" was based on a triple crown ether structure **12** while the tripod $\mathbf{13}^{9+}$ is derived from three threads each bearing the two stations. When the thread is protonated with TFA the "platform" resides over the alkylammonium moieties. Deprotonation of the thread-bound ammonium moieties with phosphazene weakens the interaction between the platform and these sites, and the platform moves to the bipyridinium units.

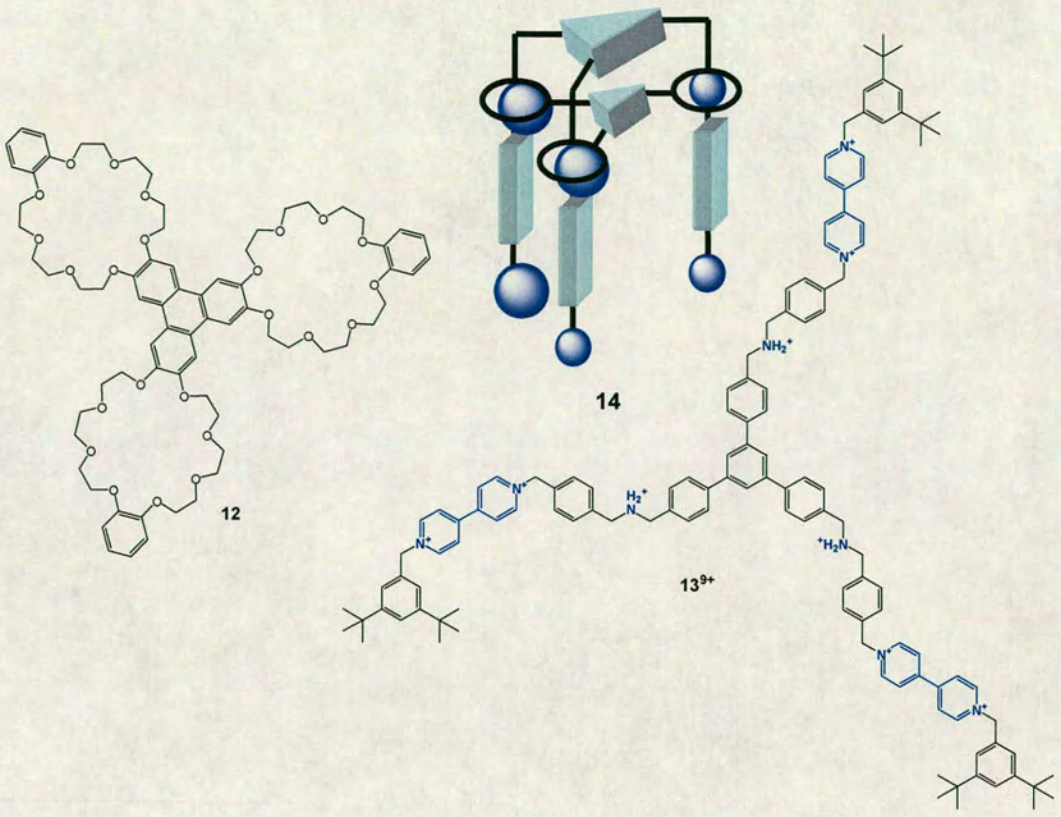


Figure 12. Molecular elevator **14** reported by Stoddart.

In 2004 Leigh and co-workers[31] synthesised [2]rotaxane **15** wherein shuttling of the macrocycle was achieved through a mechanism involving anion recognition *via* hydrogen bonding interaction (Figure 13).

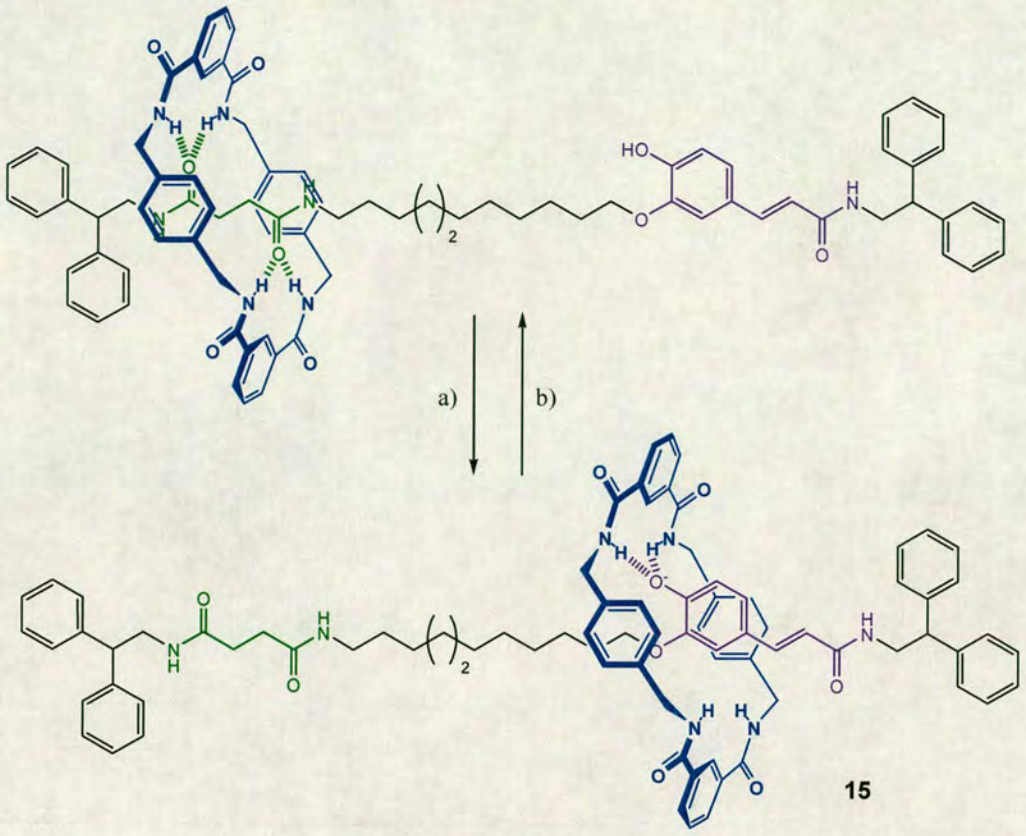


Figure 13. [2]rotaxane featuring shuttling based on anion recognition reported by Leigh and coworkers. a) Various bases; b) CF_3COOD.

In this rotaxane the thread features two stations with hydrogen bond acceptors for a tetraamide macrocycle. One station is a succinamide unit, known to be a medium to good template for the formation of the rotaxane, and the other station is a cinnamate derivative, a poor hydrogen-bond site for the tetraamide macrocycle. In the neutral state the macrocycle spends most of its time over the succinamide station (the limit of NMR reports an occupancy greater than 95%). Upon deprotonation of the phenol moiety of the cinnamate station to a phenolate anion, shuttling of the macrocycle to this station results, induced by the binding between the N-H of the tetraamide macrocycle and the phenolate anion. Shuttling occurs only when DMF-d_7 is used as solvent for this system. When deprotonation of the cinnamide unit was performed in apolar non hydrogen bond disrupting solvents, such as $CDCl_3$ and CH_2Cl_2, shuttling of the macrocycle was not observed. In these cases the thread folds over to allow the macrocycle to bind both the succinamide station and the phenolate ion. In DMF,

shuttling is observed because it is a hydrogen-bonding competing solvent. The solvent, in this case, binds the succinamide station, compensating the loss of four strong hydrogen bonds between the station and the macrocycle; such compensation is not achieved by CH_2Cl_2 or $CHCl_3$, both of which are poor hydrogen bonding accepting solvents. Protonation of the phenolate ion restores the system to its original state.

A completely different system was reported in the Leigh group.[32] Rotaxane **16** is based on a glycine-glycine station, with high affinity for the tetraamide macrocycle and a succinic amide-ester station with a lower affinity (Figure 14). In this system there is also a modified bis(2-picolyl) amino (BPA) stopper bound to the N-terminus glycine-glycine station. Shuttling is achieved by transition metals such as Cu(II) or Cd(II) ions. In the absence of any metal the macrocycle resides over the peptide template. In the presence of Cd(II) the BPA unit and one carbonyl group of the peptide moiety coordinate to the metal. However, the affinity of this site for the macrocycle is only slightly modified and only subsequent addition of a base to the system results in selective deprotonation of the NH of the peptide station, and the metal now preferentially coordinates to the BPA moiety and the carboxamido nitrogen, and in effect wraps around the station. This change results in displacement of the ring to the weaker station. Protonation of the amide nitrogen of the station restores the system to its original state.

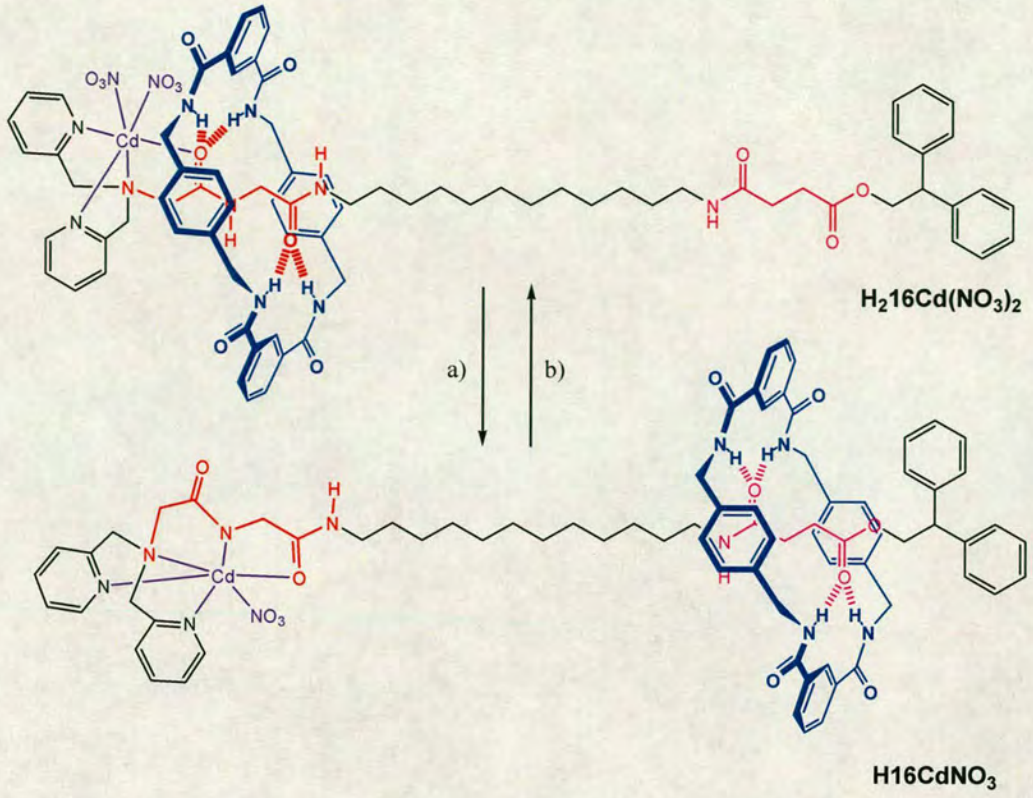


Figure 14. Another example of a shuttling achieved by removing protons reported by Leigh and coworkers. a) base; b) NH_4Cl.

1.2.2 Controlling the shuttling by adding or removing electrons.

Control of the shuttling process can be achieved by adding or removing electrons. The first redox-responsive molecular shuttle was reported by Sauvage and coworkers in 1999.[33] [2]rotaxane **17** (Figure 15) featured a thread based on a bidentate phenanthroline and a tridentate terpyridine unit and a macrocycle based on a bidentate phenanthroline ligand. The shuttle exploited the preference of Cu(I) to complex the phenanthroline ligands in a tetracordinate fashion, while Cu(II) selectively forms a complex with the phenanthroline ligand and the terpyridine unit to give a 5-coordinate complex. Thus, when the shuttle complexes Cu(I) the macrocycle and thread bidentate phenanthroline ligands coordinate the metal as seen also for [2]catenane **1**. Upon electrochemical oxidation of Cu(I) to Cu(II) the macrocycle shifts to allow the metal to coordinate the terpyrine ligand of the thread

and the phenanthroline unit of the macrocycle. This [2]rotaxane was characterised by a slow shuttling ($K = 1.5 \times 10^{-4}\ s^{-1}$) as, although not stable, the Cu(II) tetracoodinate complex initially formed was kinetically slow to exchange to the more stable co-conformation.

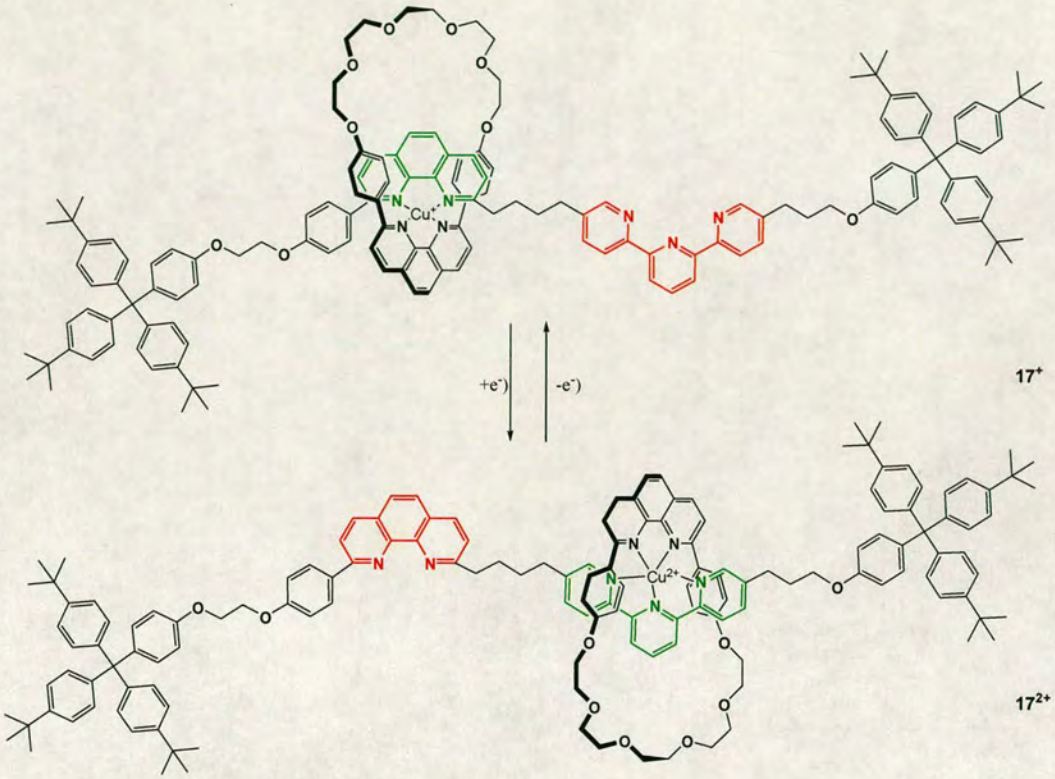


Figure 15. Electrochemical switschable molecular shuttle reported by Sauvage.

In 2001, Leigh and coworkers[34] reported another example of an electrochemically driven molecular shuttle (Figure 16). [2]Rotaxane **18** is based on a two station thread where in its resting state (shown by ^1H NMR) the macrocycle is mainly situated on the succinamide unit rather than the naphthalene derived station because the latter is a poor hydrogen bond acceptor. When electrochemically reduced (which may also be achieved photochemically)[35] the radical anion thus formed binds strongly to the tetraamide macrocycle and effects shuttling of the macrocycle towards the newly formed station.

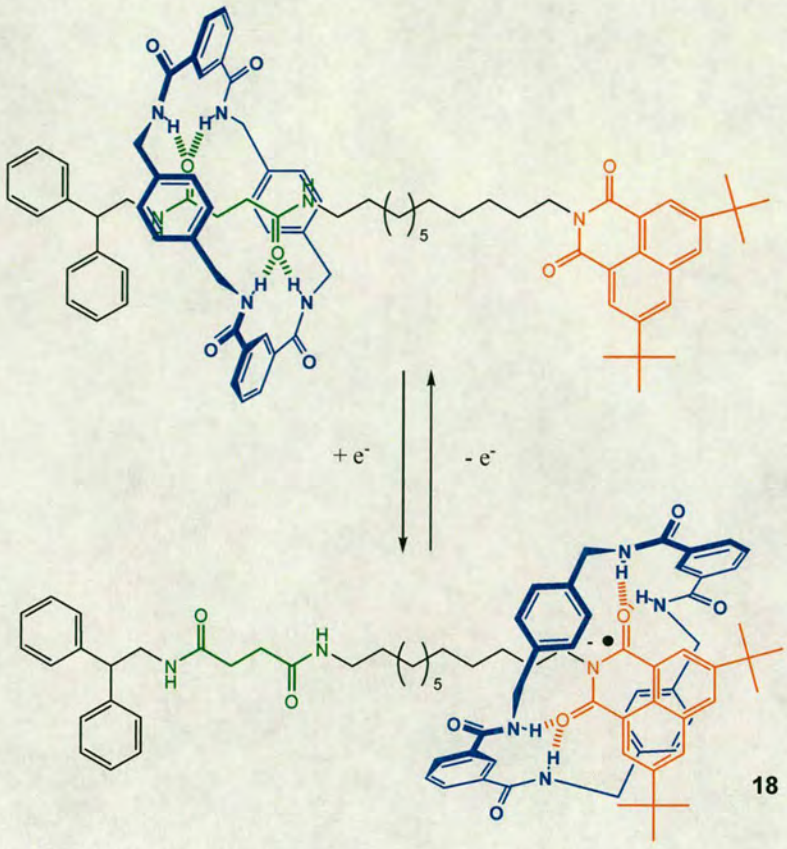


Figure 16. Electrochemical driven molecular shuttle proposed by Leigh and coworkers.

The shuttling occurs very fast (~ 50μs in THF at rt) and may be studied only by cyclic voltammetry or transient absorption spectroscopy (in case of the photochemical reduction). Oxidation of the naphthalene radical anion restores the system to its neutral form and the macrocycle shuttles back to the succinamide unit.

In 2003, Stoddart and co-workers reported electrochemically driven rotaxane, **19** (Figure 17).[36] The mechanism, in this case, involves electrostatic interaction between the ring, a cyclobis(paraquat-p-phenilene) $CBPQT^{4+}$, and the two stations of the thread, an electron rich dioxynaphthalene (DNP) unit and a redox controllable tetrathiafulvalene station (TTF). When the TTF station is in its neutral state, $CBPQT^{4+}$ displays a stronger affinity for this station. Upon oxidation of the TTF station to TTF^{2+}, the cationic $CBPQT^{4+}$ ring and the cationic station now repulse each

other and the result is the shuttling of the ring to the DNP station. The system returns to its initial state by chemical reduction of the TTF^{2+} station to neutral TTF.

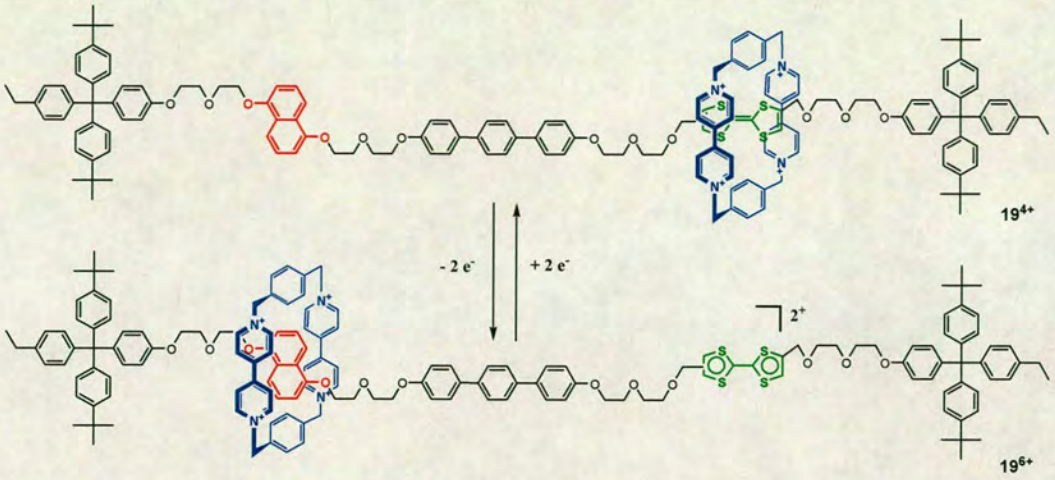


Figure 17. Redox-switchable molecular shuttle **19** reported by Stoddart and coworkers.

1.2.3 Controlling the shuttling by adding or removing metals.

Similar to the system **16** based on competitive binding of a metal ion, Leigh and coworkers[37] reported another system where the shuttling is controlled by metal coordination (Figure 18). Based on Leigh's hydrogen bonding architecture this rotaxane bears a similar BPA metal-chelating stopper and two different stations, a succinamide, with higher affinity and a succinic amide-ester, with lower affinity for the macrocycle. The special feature of rotaxane **20** is the succinamide station *directly* attached to the BPA unit; i.e. through the amide nitrogen of the succinamide moiety. When the chelating stopper is not binding any metal the macrocycle resides over the succinamide station. Coordination of transition metal ions like Cu(II) or Cd (II) by all three nitrogen atoms of the BPA group results in a forced orthogonal twisting of the pyridine "arms", causing them to enter space that is already occupied by the tetraamide macrocycle. This conformational change results in shuttling to the second station. Treatment with NaCN removes the metal ion and restores the system to its original state.

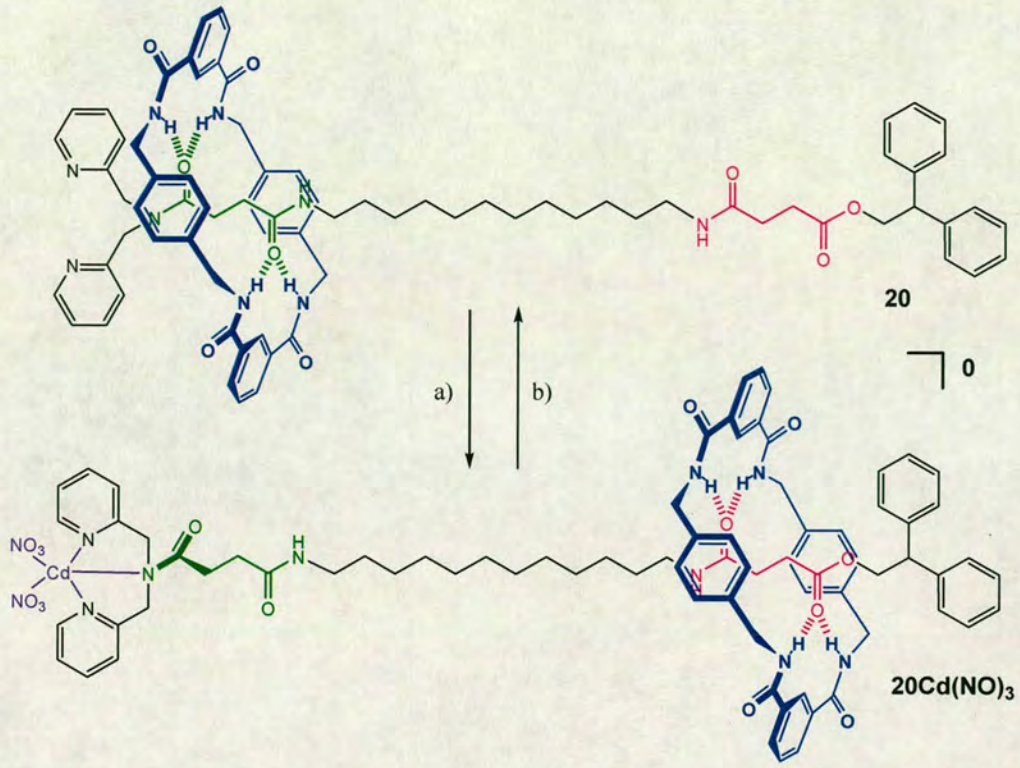


Figure 18. An allosterically regulated molecular shuttle. A) $Cd(NO_3)_2$; b) NaCN.

Sauvage and coworkers synthesized a second system where the shuttling is controlled by reversible metal coordination.[38] In this case the system is not a [2]rotaxane like those molecular shuttles described above, but consists of a linear rotaxane dimer bearing four stations (each monomer has two different stations). Sauvage and coworkers called dimer $\mathbf{21}^{2+}$ a "molecular muscle" because its function is reminiscent to the contraction of a skeletal muscle (Figure 19). In its extended form two Cu(I) ions coordinate four phenanthroline units. Removal of Cu(I) by demetalation using KCN followed by treatment with Zn(II) produces $\mathbf{21}^{4+}$. This resulting co-conformation is *contracted* compared to the Cu(I) complexed state due to the binding of Zn(II) with one phenanthroline and one terpyridine unit which are further down the thread portion of the molecule. Treatment with $Cu(CH_3CN)_4 \cdot PF_6$ restores the muscle to its starting extended conformation.

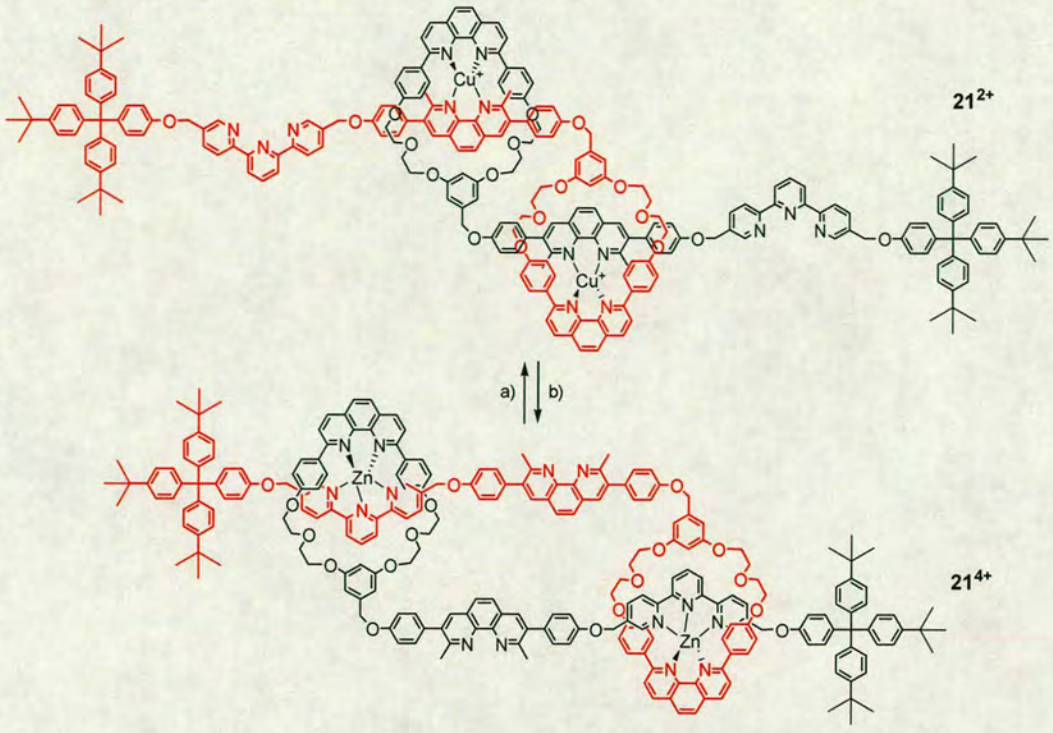


Figure 19. Sauvage's molecular muscle **21**. a) $Cu[(CH_3CN)_4]PF_6$; b) KCN and then $Zn(NO_3)_2$.

1.2.4 Controlling the shuttling by modifying or adding bonds.

In 2003, Leigh and coworkers [39] reported a molecular shuttle where motion of the macrocycle is regulated by photo and thermal stimuli. Rotaxane **22** is based on a switchable fumaramide-maleamide station and a non-switchable succinic amide-ester station separated by a lipophilic spacer of 12 methylenic groups (Figure 20). The fumaramide moiety is a superb template for the macrocycle formation (due to structural features discussed in chapter 2), in contrast to the corresponding *cis* isomer, the maleamide, which, with only two hydrogen bonds, displays very poor affinity for the macrocycle. ^{1}H NMR shows very clearly that in the initial resting state the macrocycle resides over the fumaramide station. However, irradiating with UV light (λ = 254 nm) results in isomerization of the *trans* double bond to the *cis* geometry (*cis/trans* ratio = 0.9), rendering this new station a very poor binder for the macrocycle, and shuttling of the tetraamide macrocycle is observed to the succinic

amide-ester station. Thermal (or chemical) isomerization of the double bond returns the system back to its original state.

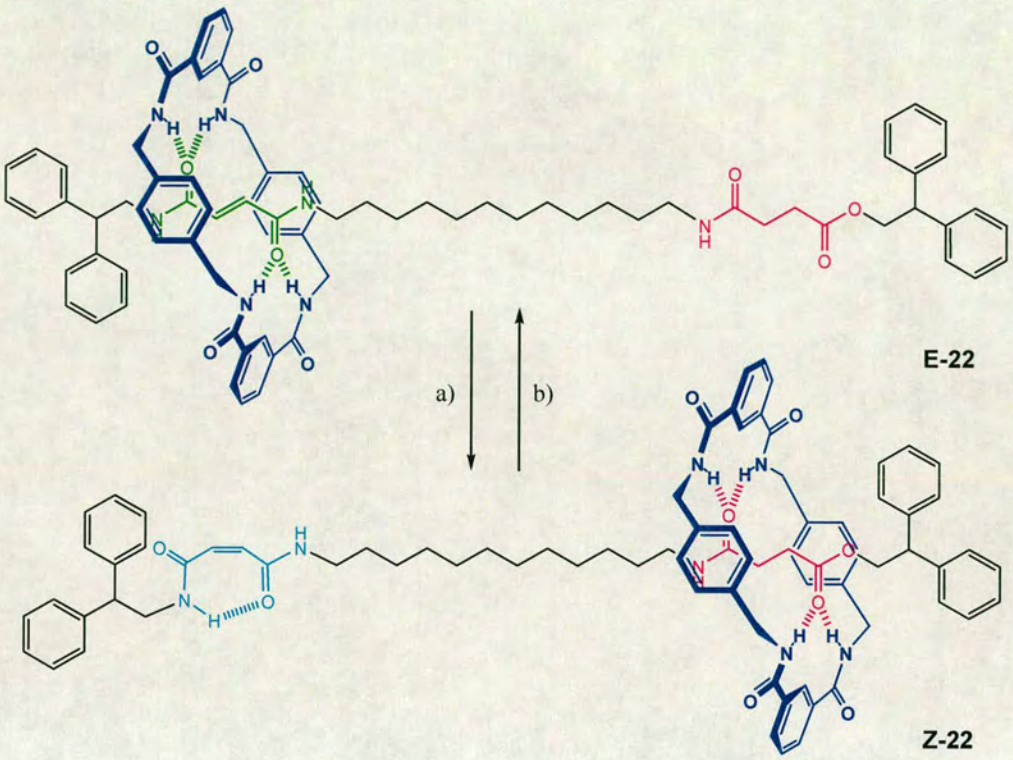


Figure 20. Leigh's light and heat switchable molecular shuttle **22**. a) λ = 254 or 312 nm; b) heat or piperidine.

Control of the shuttling has also been demonstrated by making use of Diels-Alder and retro Diels-Alder chemical reactions. [40] In molecular shuttle **E-22** displacement of the macrocycle is achieved by the sterically demanding Diels-Alder adduct formed on the fumaramide station (Figure 21).

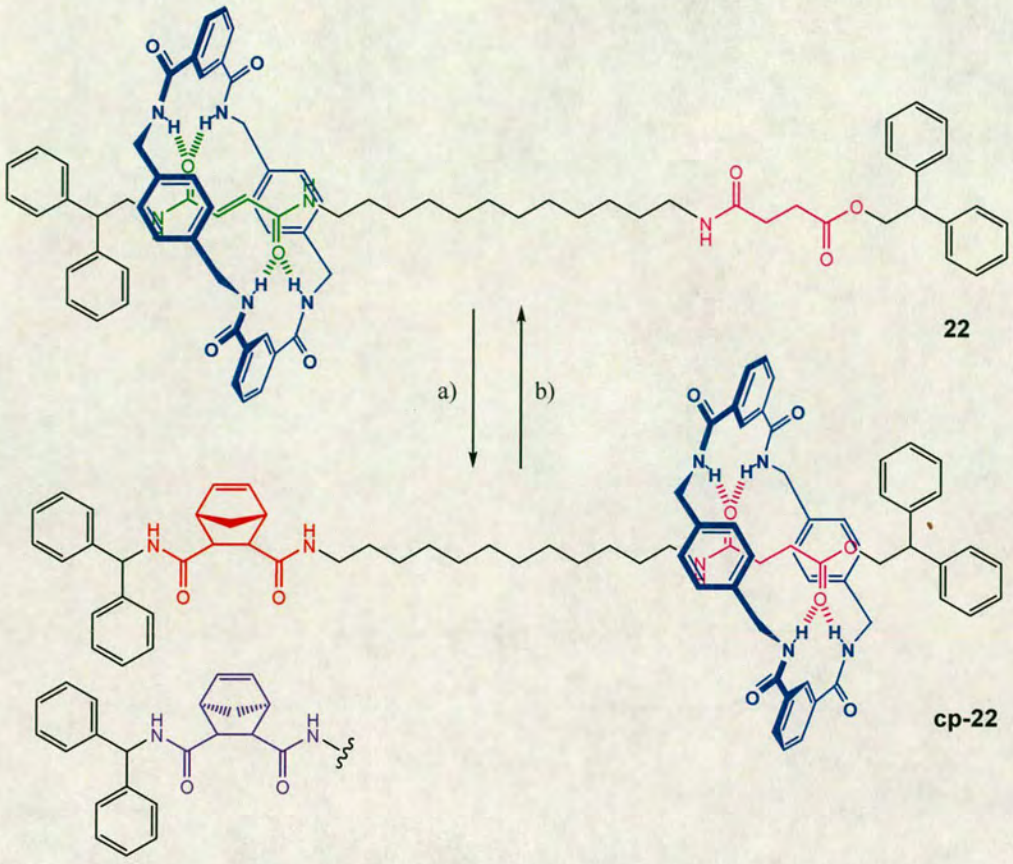


Figure 21. Shuttling through formation of covalent bonds. a) cyclopentadiene, DMSO, 80 °C, b) 250 °C, 1022 Torr, 20 min.

1.2.5 The use of controlled sub-molecular shuttling to change macroscopic properties.

The molecular shuttles described previously described are prototype molecular shuttles where the controlled translocation of the macrocycle is achieved through an external stimulus. In the following examples, the development of molecular machines where shuttling is exploited to alter the bulk chemical properties are focused on.

In 2004 Tian and co-workers [41] described a light-driven molecular shuttle in which an α-cyclodextrin (the macrocycle) shuttles between a stilbene and a biphenyl unit through alternate irradiation (Figure 22). When the stilbene unit in **23** is in a *trans* conformation, the macrocycle resides mainly over this moiety, strongly held there by

a hydrogen bond network between the cyclodextrin and the adjacent terminal isophthalic acid. Shuttling to the other station occurs after irradiation of the stilbene to the *cis* isomer due to unfavorable steric interaction between the cyclodextrin and stilbene. During their studies to isomerise the double bond, Tian and coworkers discovered that a strong hydrogen bonding network between macrocycle and stopper prevented direct irradiation. It was found to be necessary to deprotonate the carboxylic moieties of the isophthalic stopper in order to allow isomerization of the double bond. Shuttling of the α-cyclodextrin to the biphenyl station results in a 46% increase in the fluorescence intensity of the 4-amidonaphthalimide stopper moiety. The initial fluorescent intensity is restored through irradiation of the stilbene unit at 280 nm (Figure 22).

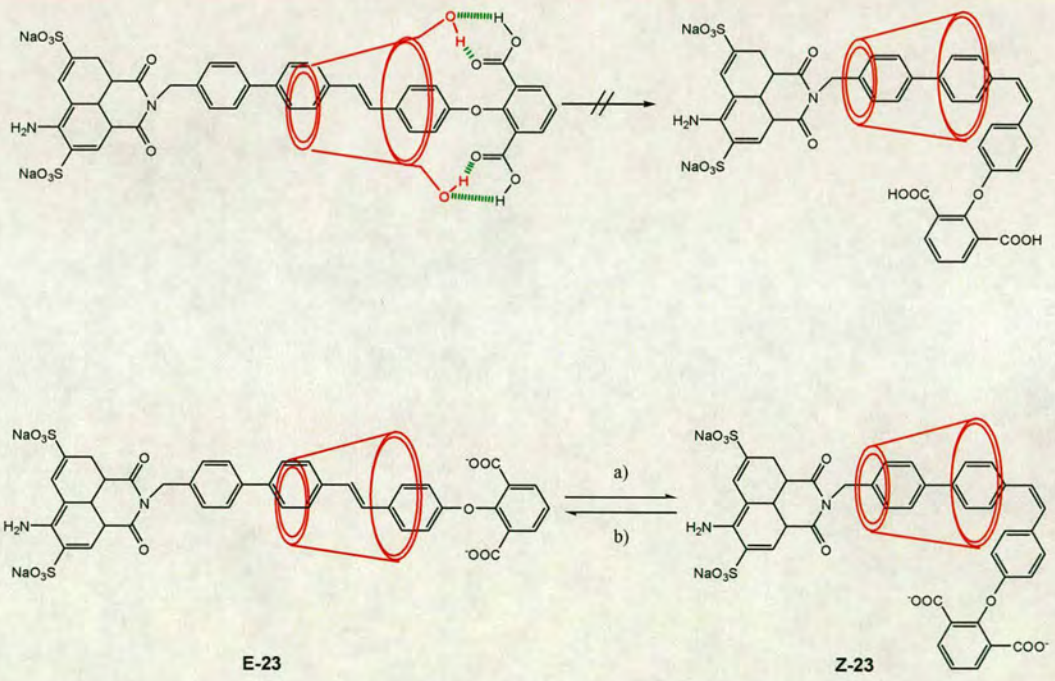


Figure 22. Tian's ligh-driven molecular shuttle **23**. a) λ = 355 nm b) λ = 280 nm.

Leigh and co-workers [42] have also described a light-driven molecular shuttle where movement of the macrocycle between the two stations switches the fluorescence on or off (Figure 23). Rotaxane **24** is comprised of the switchable fumaramide-maleamide station previously described and a glycine-glycine dipeptide motif having

intermediate binding affinity for the tetraamide macrocycle. The system is further modified by having an anthracene fluorophore bound to the peptide station, which also acts as stopper. This macrocycle bears two pyridinium ions, one on each of the isophthalamide moieties which quench the fluorescence of the anthracene unit by electron transfer. When the macrocycle resides over the fumaramide station fluorescence is observed. However, after isomerization of the double bond, the macrocycle resides predominantly over the peptide motif adjacent to the fluorophore. The pyridinium ions quench the emission almost completely. The change between the two states is so dramatic, one can observe it with the naked eye. Figure 23 shows the E and Z shuttles and the difference in fluorescence between the two states.

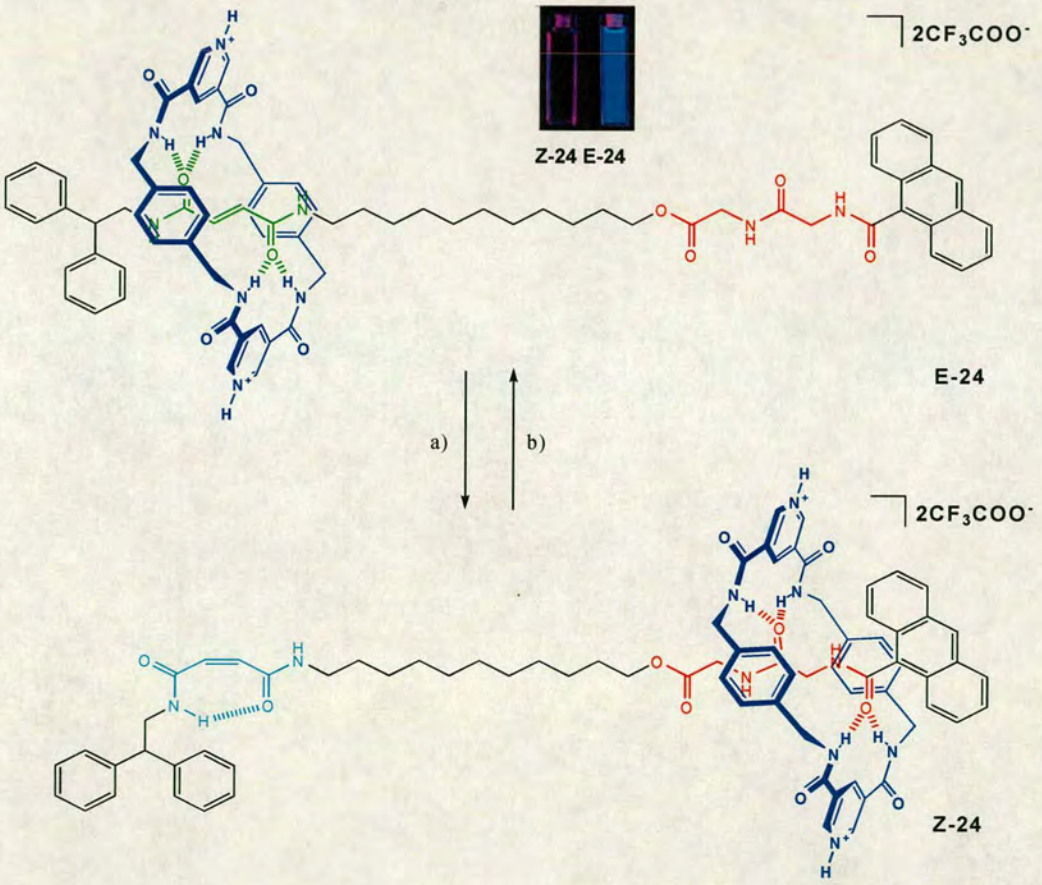
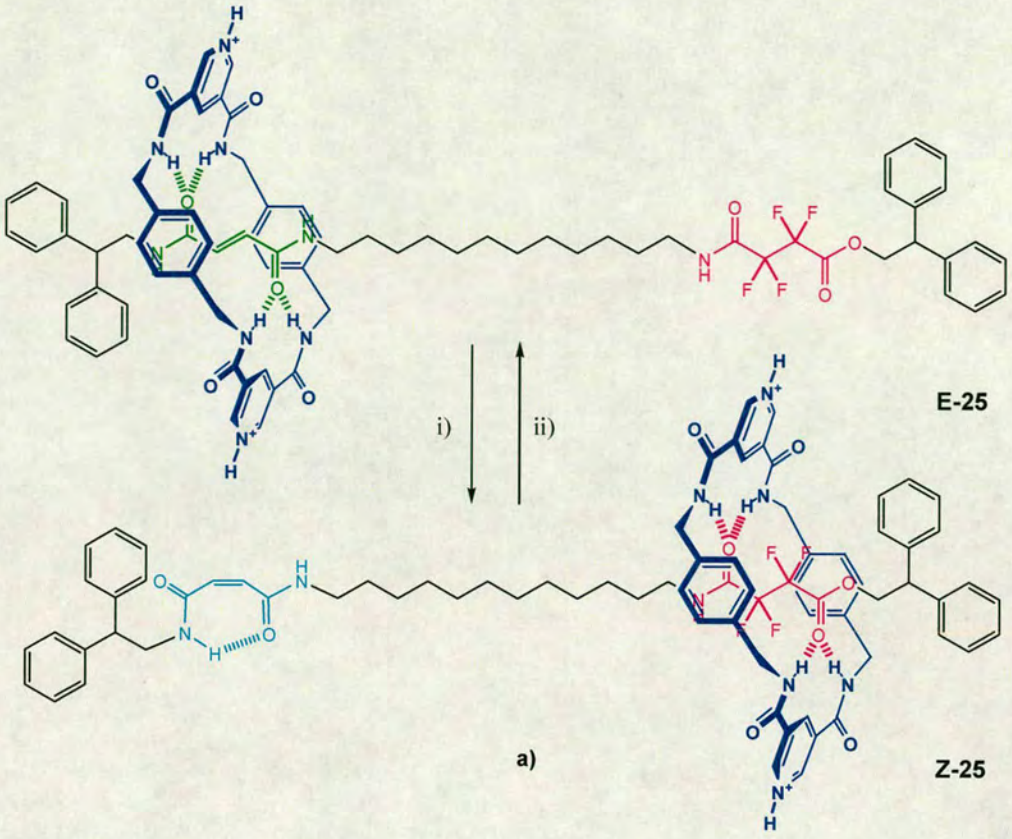


Figure 23. A fluorescence molecular shuttle reposted by Leigh and coworkers. a) λ = 312 nm; b) piperidine or heat.

In the following system, [43] macroscopic motion was achieved through the exploitation of submolecular movement in a molecular shuttle. In this case, Rotaxane **25** was used for the directional transport of an iodomethane drop across a surface, using molecular shuttle induced formation of polarophobic/polarophilic surfaces. This system is based on the familiar fumaramide-maleamide switchable station and a second station bearing a fluoroalkane unit having intermediate affinity for the macrocycle (Figure 24a). The tetraamide macrocycle bears two pyridinium ions which allow the rotaxane to be physorbed onto a Self Assembled Monolayer (SAM) of mercapto undecanoic acid (MUA) on a gold surface (Figure 24b). Initially the macrocycle resides over the fumaramide station, leaving the fluorinated station exposed to the surrounding environment and creating an overall polarophobic surface. Isomerization of the double bond induces shuttling of the macrocycle to the fluorinated side of the rotaxane, masking it and creating an overall polarophilic surface. The contact angle of an iodomethane drop with the surface changes in response to the isomerization process. Movement of a microlitre droplet up a 12 degree incline was achieved.



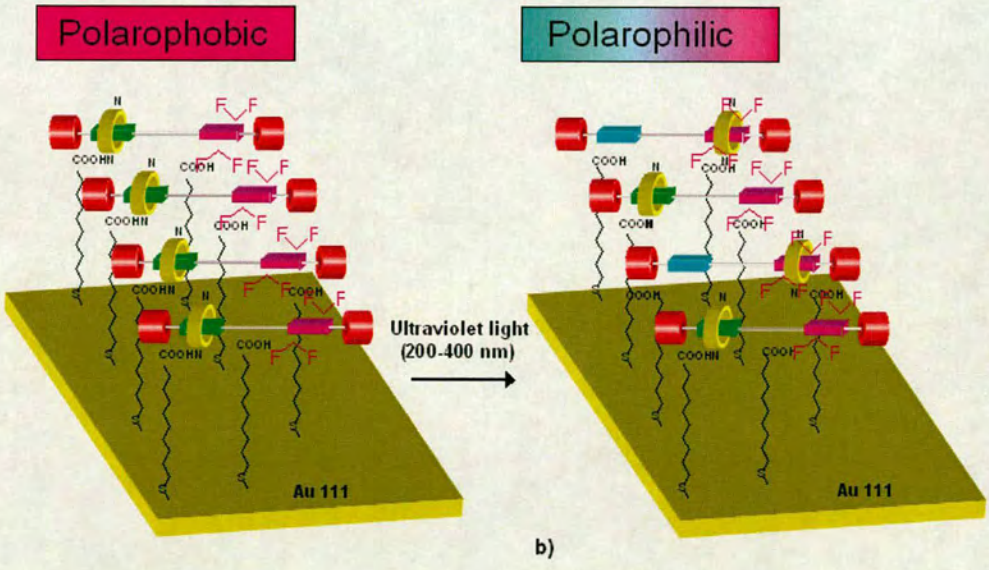
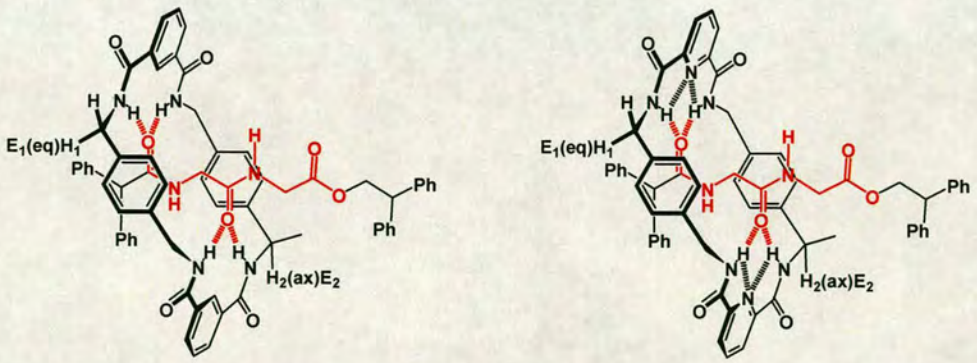


Figure 24. a) Leigh's [2]rotaxane bearing a fluoroalkane unit. i) λ = 254 or 312 nm; ii) heat or piperidine; b) Cartoon representing the formation of the Polarophobic and Polarophilic surfaces.

1.3 Control of the Pirouetting of the macrocycle.

One method of studying symmetric benzylic amide macrocycles is to follow changes in the ^{1}H NMR of the benzylic protons. The benzylic amide macrocycle usually adopts a chair-like configuration, meaning that one of the benzylic protons resides in an axial conformation and the other in an equatorial conformation (Figure 25). A 180° degree rotation around the thread followed by a chair-chair flip results in an interconversion of the axial and equatorial protons, while in a full 360° rotation the interconversion happens twice. Variable temperature NMR (VT-NMR) techniques, studying the temperature coalescence of these protons therein, may be used to determine this exchange process. In the room temperature ^{1}H NMR spectra of glycylglycine-based [2]rotaxane **26** in chloroform, one observes the fewest possible signals for the macrocycle indicating the axial-equatorial exchange process is rapid on the NMR timescale. [44] However, in the pyridyl-2-6-dicarbonyl-based macrocycle **27** a reduction of the pirouetting rate occurs due to the stronger hydrogen bonds between the macrocycle and the thread and more peaks are seen in the NMR spectra of this system under the same conditions.

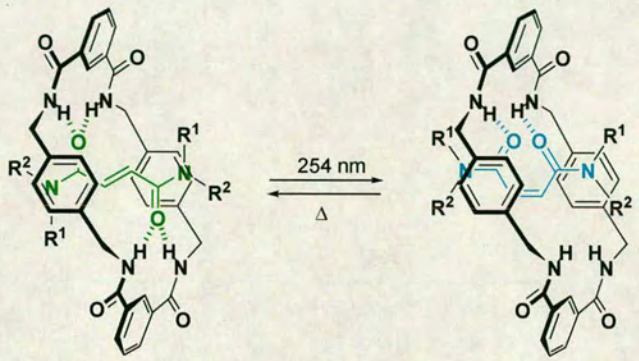


26 **27**

Figure 25. Rotaxanes **26** and **27**.

Pirouetting of the benzylic amide macrocycle may also be controlled through E/Z photo-isomerization.[45] As described above, the fumaramide moiety may be isomerized to the maleamide with UV light (Figure 26). The change of geometry

induced in the fumaramide-based rotaxane after isomerization, from a system with four strong bifurcated hydrogen bonds to one with two, reduces the strength of the interaction between the benzylic amide macrocycle and this station, with a consequent increase in pirouetting speed. To study this phenomenon further, a series of rotaxanes were synthesized and it was observed that the pirouetting speed could be increased by six orders of magnitude in this manner. Reduction of the speed was achieved by re-isomerization of the Z isomer **28** to the E **5** by heating the solution.



5: R^1= H, R^2 = CH_2CHPh_2
5B: R^1 = Me, R^2 = CH_2CHPh_2
5C: R^1 = R^2 = $CH_2CO_2CH_2Ph$

28

Figure 26. *trans* Fumaramide rotaxane **5** and *cis* maleamide rotaxane **28**.

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2 Synthesis of functionalized [2]rotaxanes

2.1 Introduction

In the previous chapter general methods were discussed for the preparation of interlocked architectures using non covalent interactions.[1, 2] In each of these protocols the formation of strong hydrogen bonding interactions induces the preorganisation of thread and macrocycle components which upon further derivatisation leads to the formation of the interlocked product.[3, 4] The ability of the thread and its templating unit **T** (Figure 1) to coordinate the incoming molecule **29** via hydrogen bonding interactions allows the formation of weaker secondary interactions (Van der Waals forces, π-stacking) which enhance the yield of the reaction.[5] Without the thread, linear oligomers and catenanes are formed as **29** tends to adopt a linear conformation as a consequence of the predisposition to a *syn-anti* conformation of the aromatic 1,3-diamide.

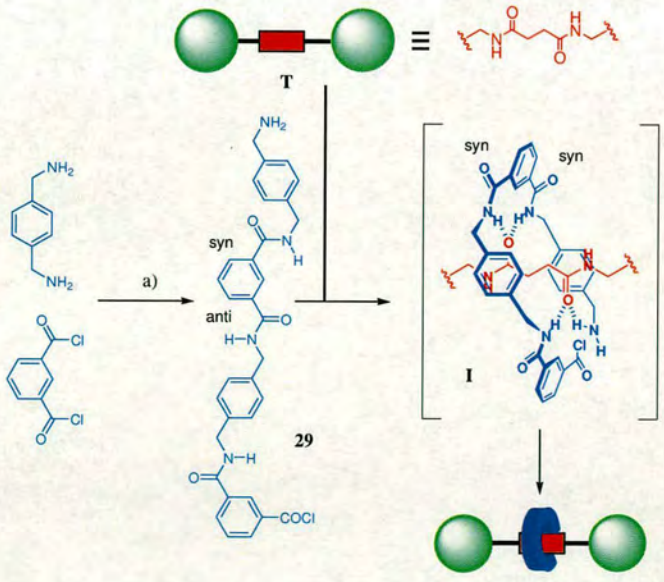


Figure 1. Rotaxane formation with succinamide based hydrogen-bond template. a) Isophthaloyl dichloride, xylylene diamine, Et_3N, $CHCl_3$.

Under rotaxane-forming conditions, in the presence of a thread, strong bifurcated hydrogen bonding interactions between the station and macrocycle constituents allow **29** to change to a *syn-syn* conformation (**I**), promoting closure of the macrocycle around the thread. Even though this succinamide motif is a good template for the benzylic amide macrocycle, the fumaramide template typically gives the highest yield of rotaxane, with yields of up to 97%.[6] This excellent templating effect can be explained by examining the structure of the fumaramide motif and its preorganizatiing influence on the macrocycle precursor:

- the hydrogen bonding accepting groups are held in a fixed *transoid* arrangement, at an ideal distance to template formation of the benzylic amide macrocycle (1.98 Å and 2.06 Å)
- the rigidity of the trans double bond reduces torsional freedom and does not allow the formation of intramolecular hydrogen bonds

The hydrogen-bond basicity of the functional group also affects the template efficacy (fumaramide is better than corresponding fumaramide-ester template).[6] These combined template properties of the fumaramide motif lead to the interlocked structure **5** stabilized by four bifurcated intercomponent hydrogen bonds between the N-H protons of the macrocycle and the two carbonyl groups of the fumaramide thread (Figure 2). Also, secondary π-π interactions are observed in the solid structure.

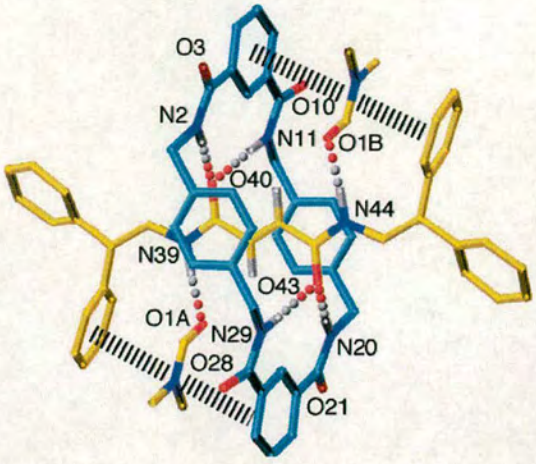
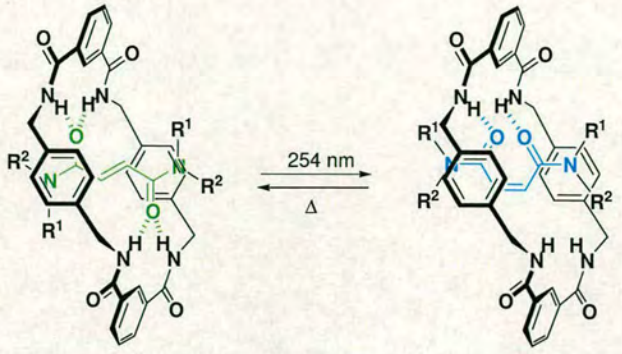


Figure 2. X-ray structures of the prototype [2]rotaxane **5** based on a fumaramide group.[6]

When rotaxane **5** is irradiated with UV light (254 nm), isomerization to the corresponding maleamide-containing rotaxanes **28** is observed (Figure 3). This isomerization reduces the number of intercomponent hydrogen bonds from four to two; unsurprisingly the affinity of the macrocycle for the maleamide station is much less than the corresponding fumaramide (Figure 3). Furthermore, the five component clipping reaction does not work directly with the maleamide thread,[7] because of fewer hydrogen bonds and the absence of the transoid arrangement found in the fumaramide group. Indeed, it was shown[7] that the maleamide [2]rotaxane **28** can be only obtained from isomerization of the fumaramide rotaxane **5**.

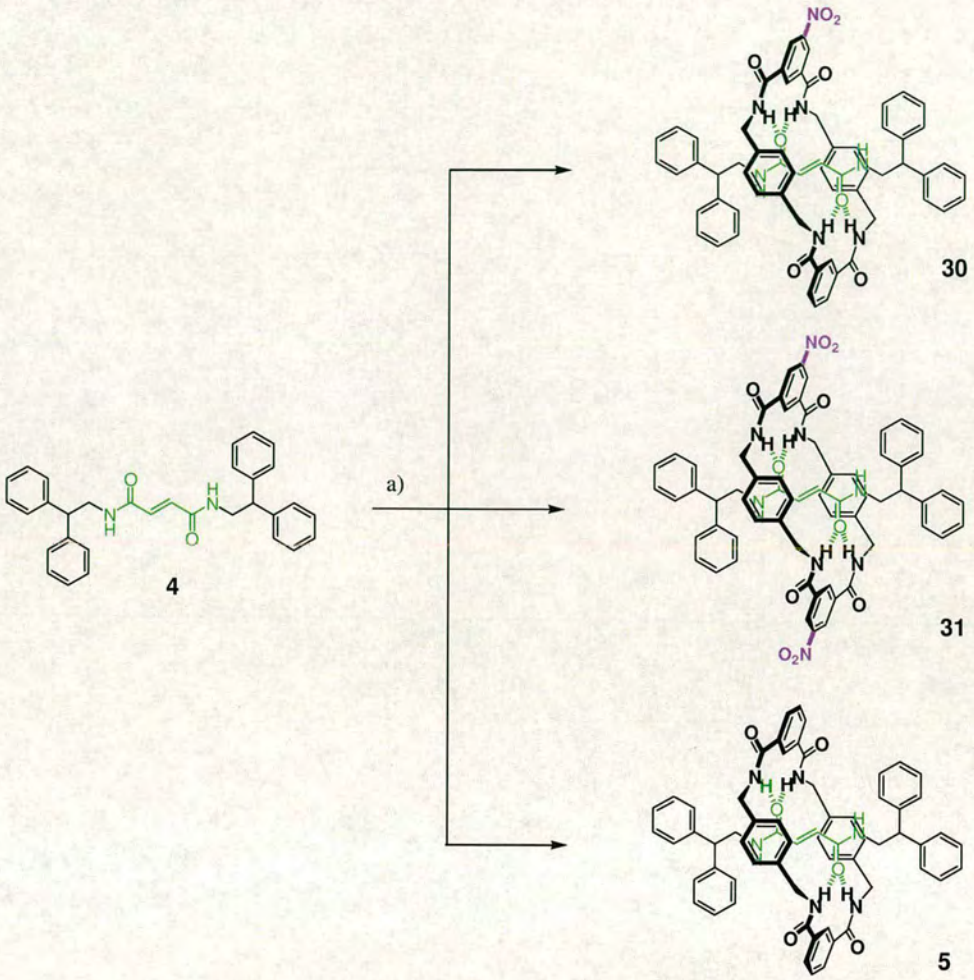


5: R^1= H, R^2 = CH_2CHPh_2
5B: R^1 = Me, R^2 = CH_2CHPh_2
5C: R^1 = R^2 = $CH_2CO_2CH_2Ph$

28

Figure 3. Synthesis of the maleamide [2]rotaxane **28** from fumaramide [2]rotaxane **5**.

In the Leigh group, rotaxanes have been synthesized by a five-component hydrogen bond directed clipping reaction with good to excellent yields using peptides,[8, 9] nitrones,[10] diamides and esters[11] as templates. These [2]rotaxanes possess a highly symmetrycal macrocycle. However, in order to functionalize the rotaxane via the macrocycle it would be advantageous to introduce one functional group to the macrocycle. To date desymmetrization of the macrocycle component has resulted in much lower yields of rotaxanes, primarily a result of the statistical method employed in their synthesis. For example, the mono-nitro rotaxane **30** was isolated in just 27% yield after the simultaneous addition of xylylene diamine, isophthaloyl dichloride, 5-nitro-isophthaloyl dichloride to the fumaramide thread **4**.[12] While the statistical reaction can produce up to 50% of the desired desymmetrized rotaxanes, part of the difficulty comes in separating the mixture of the three different statistical products (like those shown in Scheme 1), which usually have similar properties. The X-ray structure of **30** is shown in Figure 4.



Scheme 1. Statistical synthesis of a [2]rotaxane with an unsymmetrical macrocycle. Reagents and yields: a) xylylene diamine, isophthaloyl dichloride, 5-nitro-isophthaloyl dichloride, Et_3N, $CHCl_3$; 27%, 29%, 31% of **30, 31** and **5** respectively.

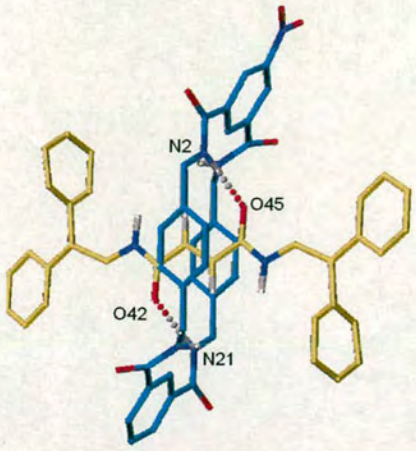


Figure 4. *X*-ray crystal structure of the fumaramide-based [2]rotaxane **30** with unsymmetrical macrocycle crystallized from DMF. The carbon atoms of the macrocycles are shown in blue, carbon atoms of the threads in yellow, oxygen atoms in red and nitrogen atoms in dark blue. The amide and alkene hydrogen atoms are shown in white, all others are removed for clarity.[12]

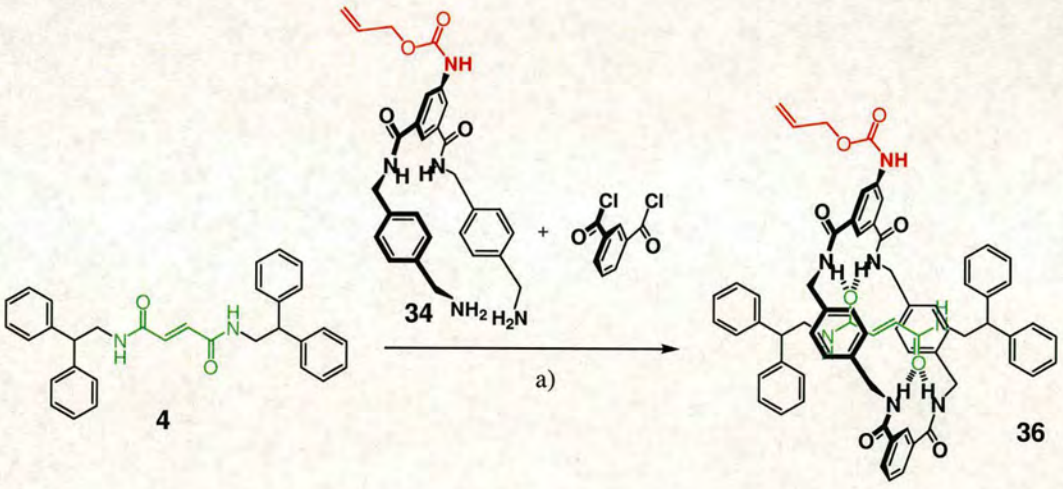
The statistical approach to rotaxanes with monofunctionalised macrocycle components can be tolerated when the thread precursors can be readily obtained in significant amounts. However, thread precursors for most two-station molecular shuttle-type rotaxanes are not simply prepared in a couple of steps, and this statistical approach not only results in lower rotaxane yield, but significantly, the thread is often lost to unwanted symmetrical [2]rotaxanes. A new approach for the synthesis of monofunctionalised rotaxanes that proceeds in high yields, and also allows the recovery of the thread will be demonstrated in the next section.

2.2 A non-statistical route to [2]rotaxanes based on a monofunctionalised macrocycle

In this chapter a convenient, non-statistical synthesis of [2]rotaxanes with unsymmetrical macrocycles is reported using three different procedures. All three syntheses involve the use of a pre-formed macrocycle precursor, which can be referred to as a "¾-macrocycle".

2.2.1 Synthesis of a [2]rotaxane using a preformed macrocycle precursor bearing a N-Alloc protected amine.

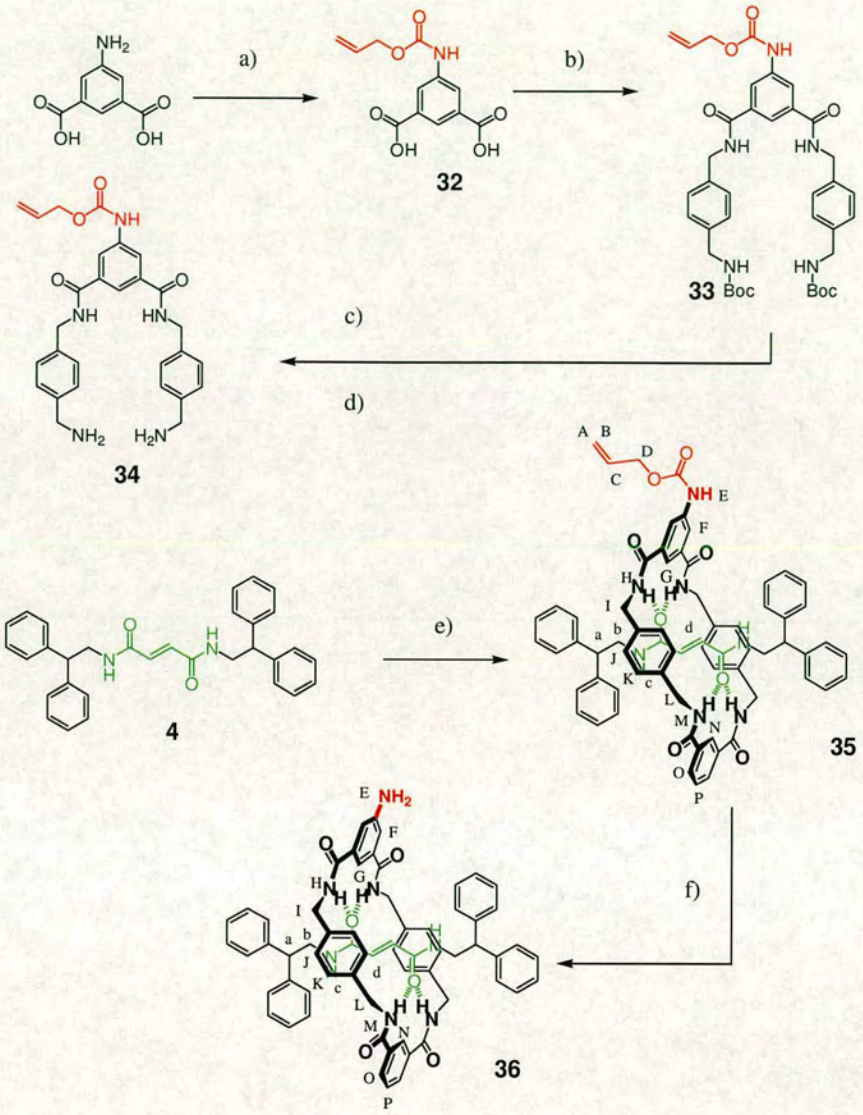
The general, but non-statistical approach to rotaxanes with monofunctionalized macrocycles, as described in this thesis, is outlined in Scheme 2. This approach is based on the reaction of a preformed, and appropriately functionalized, diamino ¾-macrocycle, **34**, with isophthaloyl dichloride in the presence of a thread molecule. Although it is practically more complex to create a functionalized ¾-macrocycle, rather than introduce monofunctionality via the isophthaloyl dichloride component, the outlined approach can be readily adopted to give macrocycles with double but different functionality. Either way, these non-statistical approaches lead to only one rotaxane product, and so any unreacted thread can be recovered and recycled.



Scheme 2. Synthesis of [2]rotaxane **35**; a) Et_3N, anhydrous $CHCl_3/CH_3CN/DMF$ (90/8/2), RT, isophthaloyl dichloride was added to a solution of **4** and **34**.

5-Aminoisophthalic acid was chosen as the building block for the ¾-macrocycle component because the free amine can easily be used for post modification of the rotaxane. An early example from Rebek and coworkers showed a similar approach with an *N*-protected amine bearing a long alkyl chain (used to improve the solubility of the

macrocycle component).[13] A similar strategy was adopted in the synthesis of the asymmetric rotaxane **35** (Scheme 3). 5-Aminoisopthalic acid was protected with an Alloc group, by reaction with allyl chloroformate giving **32** in quantitative yield (Scheme 3, step a). Diacid, **32** was coupled with two equivalents of mono-Boc protected p-xylylenediamine using EDCI and HOBt in DMF to give **33** in a 75% yield (Scheme 3, step b). Removal of the Boc groups from **33** in CH_2Cl_2/TFA and subsequent neutralization of the primary ammonium salts using $NaHCO_3$ (aq) gave the "¾-macrocycle" **34** in a 68% yield (Scheme 3, step c and d) which showed a low solubility in both CH_2Cl_2 and $CHCl_3$, normally the solvents of choice for rotaxane-forming reactions. However, macrocyclization of **34** and isophthaloyl chloride in the presence of the the fumaric thread **4** in a mixed solvent system - $CHCl_3/CH_3CN$/DMF (90/8/2) - gave [2]rotaxane **35** with a moderate 31% yield (Scheme 3, step e), despite the presence of hydrogen-bonding solvents and only two equivalents of ¾-macrocycle, **34**. The use of DMF and a higher amount of solvents were necessary to solubilize **34** (0.6 mmol of thread **4** and 1.2 mmol of **34** were dissolved in 200 mL of solvent). As a comparison, the standard five component clipping reaction is carried out in the presence of 4 eq. of xylylene diamine and isophthaloyl dichloride, dissolving 1 mmol of fumaramide thread in 100 mL of $CHCl_3/CH_3CN$ (9:1). With this approach (scheme 3, step e), only a low amount of [2]catenane was observed (5%), and more importantly unreacted thread, **4**, was fully recovered. Removal of the *N*-Alloc group using Pd(0) and dimedone in THF gave monoamino functionalized [2]rotaxane **36** in a good 89% yield.



Scheme 3. Synthesis of the Alloc-protected [2]rotaxane **35** and amino mono-functionalized [2]rotaxane **36**. Reagents and yields: a) allyl chloroformate, NaOH 1M, dioxane, 97%; b) EDCI.HCl, HOBt, Et_3N, mono-Boc protected p-xylylenediamine, DMF, 75%; c) CH_2Cl_2/TFA (9:1); d) $NaHCO_3$ (aq), CH_2Cl_2, 68% (over two steps); e) **34**, isophthaloyl dichloride, Et_3N, $CHCl_3/CH_3CN$/DMF (90:8:2), 31%; f) $Pd(PPh_3)_4$, dimedone, THF, 89%.

2.2.1.2 Characterization of rotaxanes 35 and 36

The ^{1}H NMR (400 MHz, 298K) spectra of thread **4** and [2]rotaxanes **35** and **36** in DMSO-d_6 is shown in Figures 5, 5a and 5b respectively. Rotaxane formation can be diagnosed by comparing the chemical shifts of thread and rotaxane. For instance the *E*-alkene protons of the thread are the most influenced by the xylylene aromatic unit of the macrocycle and consequently these alkene protons are shifted upfield by 1.14 ppm upon rotaxane formation. A small downfield shift is observed for the NH protons of the thread due to the hydrogen bonding between the carbonyl groups of the macrocycle and the NHs of the thread. After removal of the Alloc protecting group an upfield shift is observed for the protons belonging to the 5-aminoisophthalic moiety due to *ortho* substituent effects.

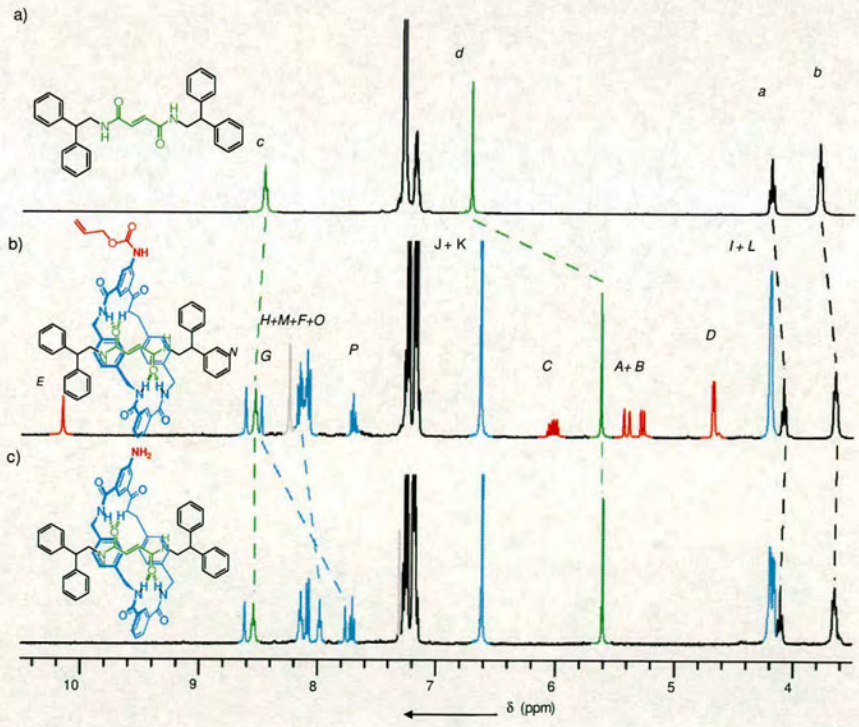


Figure 5. ^{1}H NMR [400 MHz, 298 K, DMSO- d_6] spectra of (a) thread **4**, (b) rotaxane **35** and (c) rotaxane **36** (for hydrogen labels refer to scheme 3).

The solid state structure of **35** from crystals grown in DMF/Et_2O was obtained (Figure 6). The crystal structure shows four sets of hydrogen bonds between the amide group of the macrocycle and the carbonyl group of the thread. The macrocycle adopts the typical chair conformation frequently observed in benzylic amide rotaxanes.

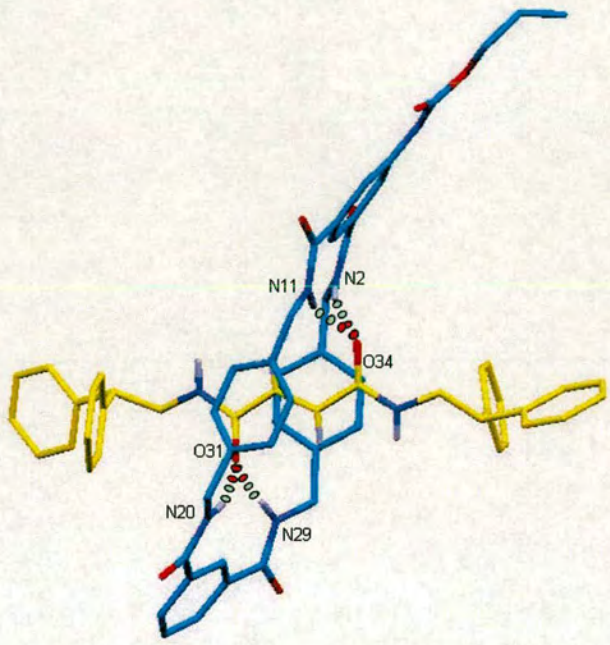
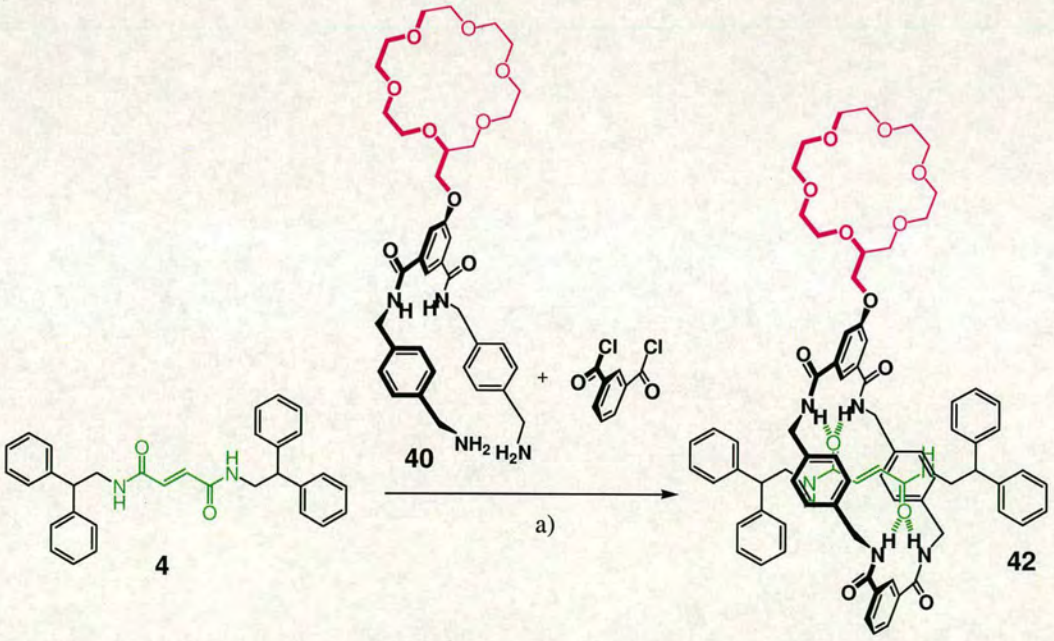


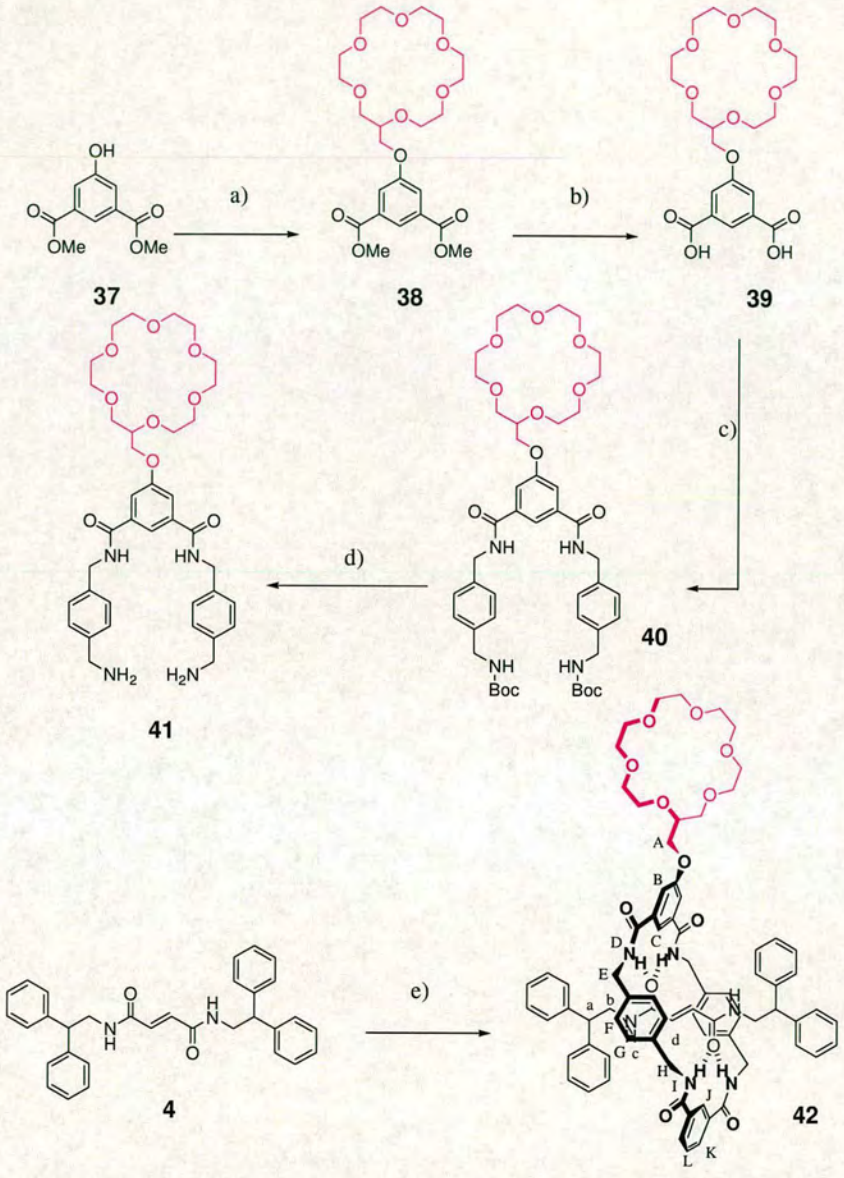
Figure 6. X-ray crystal structures of the fumaramide-based [2]rotaxane **35** crystallised from DMF/Et_2O (diffusion). The carbon atoms of the macrocycles are shown in blue, carbon atoms of the threads in yellow, oxygen atoms in red and nitrogen atoms in dark blue. The amide and alkene hydrogen atoms are shown in white while all others are removed for clarity. Intramolecular hydrogen bond distances (Å): a) O34-HN2 = 2.804, O34-HN11 = 2.053, O31-HN20 = 2.405, O31-HN29 = 1.983.

2.2.2 Synthesis of a [2]rotaxane based on an unsymmetrical macrocycle using a functionalized "¾-macrocycle"

In this section the synthesis of a [2]rotaxane with a macrocycle functionalized with a 18-crown-6 ether is discussed. The crown ether is a potentially interesting group to incorporate into rotaxanes because of its powerful ability to complex cations such as K^+ and Na^+,[14] and its incorporation into two-station [2]rotaxanes as potential anion transporters within membrane bilayers is discussed in the next chapter. The synthesis of a rotaxane in which the macrocycle was monofunctionalized with a crown-ether group is outlined in Scheme 4. This synthesis uses the same strategy for monofunctionalization (i.e. from a preformed ¾ - macrocycle component) as discussed in the previous section.



Scheme 4. Synthesis of [2]rotaxane **42**; a) Et_3N, anhydrous $CHCl_3/CH_3CN$/DMF (85/10/5), RT, isophthaloyl dichloride was added to a solution of **4** and **40**.



Scheme 5. Synthesis of a functionalized [2]rotaxane. a) 18-crown-6-methanol, DIAD, PPh_3, THF, 81%; b) NaOH (aq) THF, then HCl/dioxane, 96%; c) EDCI.HCl, HOBt, Et_3N, mono-Boc protected p-xylylenediamine, DMF, 64%; d) CH_2Cl_2/TFA (9:1), then CH_2Cl_2, diethylaminomethyl-polystyrene resin, 81%; e) **41**, isophthaloyl dichloride, Et_3N, $CHCl_3/CH_3CN$/DMF (85/10/5), 10%.

The dimethyl ester derivative of 5-hydroxy isophthalic acid was functionalized with an 18-crown-6 ether via Mitsunobu reaction giving **38** in a good 81% yield (Scheme 5, step

a). Saponification of the diester was performed using NaOH followed by acidic workup in dioxane to furnish compound **39** (Scheme 5, step b), which was then subjected to an EDCI coupling in the presence of HOBt with two equivalents of mono-Boc protected p-xylylenediamine in DMF to give the di-Boc protected ¾-macrocycle **40** in a 64% yield (Scheme 5, step c). Removal of the boc groups was performed as previously described but instead, a diethylaminomethyl-polystyrene resin was used to neutralize the ¾-macrocycle ammonium salts using CH_2Cl_2 as solvent to give **41** in 81% yield (Scheme 5, step d). Macrocyclization of **41** and isophthaloyl chloride around the fumaric thread **4** in $CHCl_3/CH_3CN$/DMF (85/10/5) yielded [2]rotaxane **42** in a 10% yield (Scheme 5, step e). A small amount of macrocycle and [2]catenanes was also obtained (5%) and the thread was fully recovered. This yield is low compared to the previous synthesis involving a ¾ - macrocycle component (Scheme 3, step f), for which there are several plausible explanations. This preformed macrocycle functionalized with the crown ether, **41**, is less soluble in chloroform than the previous alloc protected analogue **34**. Due to this a higher amount of hydrogen bond disrupting solvents was required for this synthesis (5% of DMF and 10% of CH_3CN), limiting the powerful template affinity between the thread and macrocycle components. Furthermore the 18-crown-6 ether itself is not only a strong hydrogen bond acceptor but it is also well known to give complexes with ammonium ions,[15] (the ionic diameter of NH_4^+ differs only by 0.20 Å from K^+) suggesting it may bind protonated amines reducing the number of available free amines needed for the macrocycle formation around the thread.

2.2.2.1 Characterization of [2]rotaxane 42

^{1}H NMR (400 MHz, 298K) spectra of thread **4** and [2]rotaxane **42** in DMSO- d_6 show the typical upfield shifting of the *E*-alkene protons of the thread (1.02 ppm), and a small downfield shifting for the NH protons of the thread (Figure 7a and 7b).

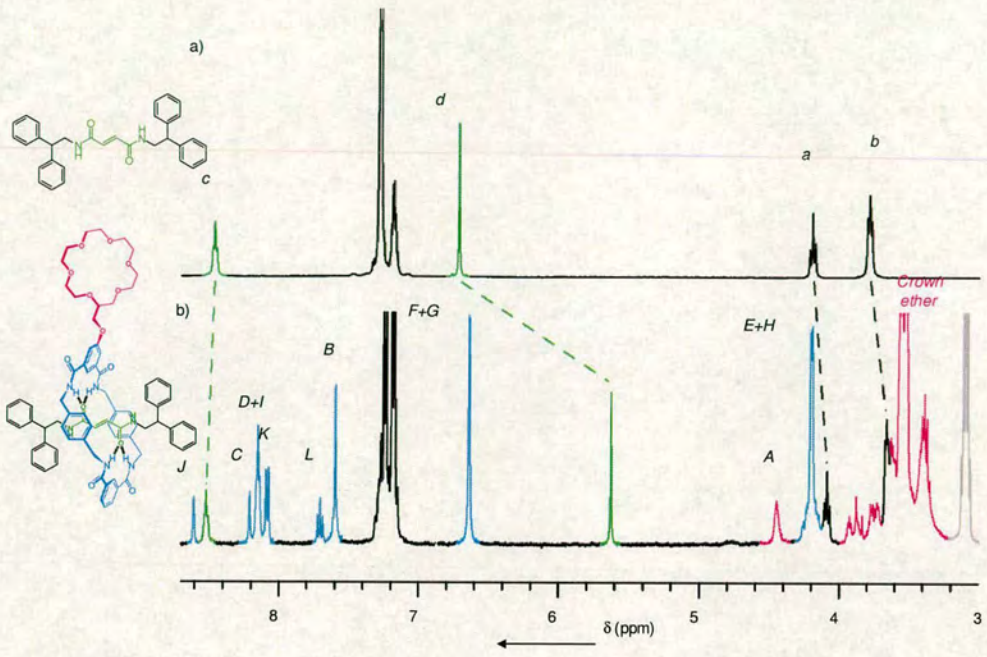
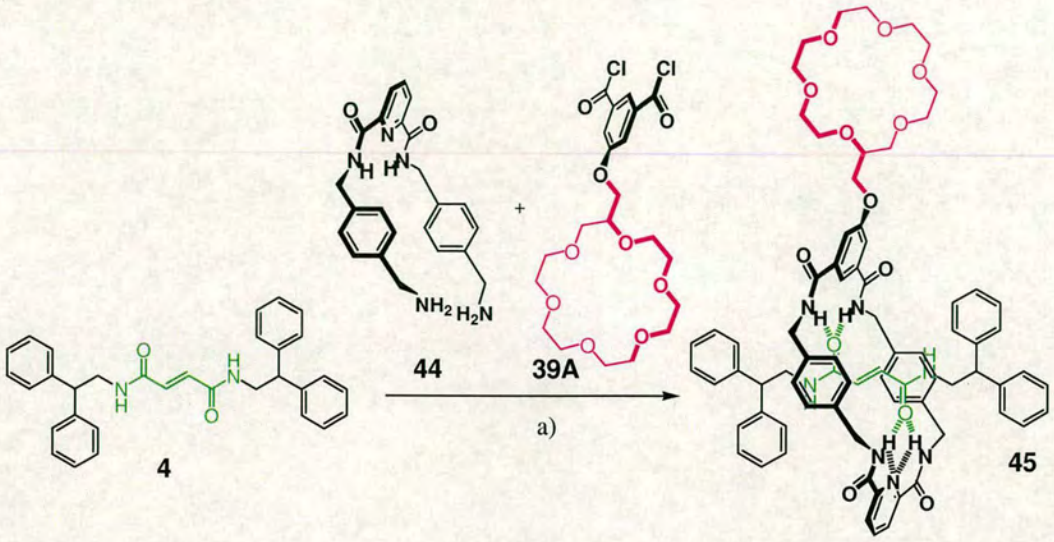


Figure 7. ^{1}H NMR [400 MHz, 298 K, DMSO- d_6] spectra of (a) thread **4** and (b) rotaxane **42** (for hydrogen labels refer to scheme 3).

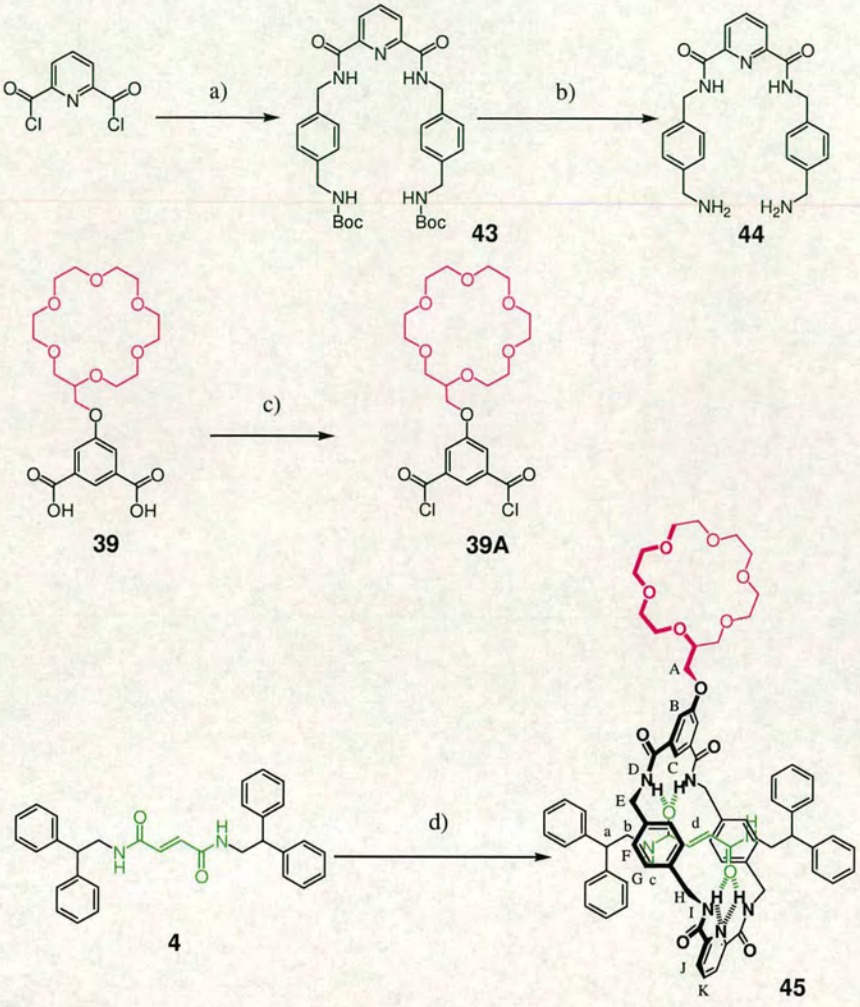
2.2.3 Synthesis of a monofunctionalized [2]rotaxane using a non functionalized ¾-macrocycle

One of the main practical difficulties encountered during both previous syntheses (sections 2.2.1 and 2.2.2) was the low solubility of the preformed ¾-macrocycles in halogenated solvents. In this section the synthesis of a more soluble analogue bearing an endotopic nitrogen atom is reported (Scheme 6). The major benefit of this approach over those previously described is that this is significally more convergent. In other words, from a single preformed ¾-macrocycle many different mono-functionalised rotaxanes can be easily obtained by reacting with the appropriate 5-substituted isophthaloyl component.



Scheme 6. Synthesis of [2]rotaxane **45**; a) Et_3N, anhydrous $CHCl_3$, RT, **39A** was added to a solution of **4** and **44**.

The synthesis of an endotopic nitrogen bearing, ¾ macrocycle, **44**, commenced from 2,6-pyridine dicarbonyl dichloride. This was reacted with with mono-Boc protected p-xylylenediamine in $CHCl_3$ to give compound **43** in 65% yield (Scheme 7, step a). Removal of the boc groups from **43** and then neutralization of the quaternary ammonium salt using aqueous NaOH gave compound **43** in high yield (Scheme 7, step b). Macrocyclization of **44** and the dichloride **39A**, [prepared from **39** using $SOCl_2$ and $(COCl)_2$ (Scheme 7, step c)], in the presence of the fumaric thread **4** (Scheme 7, step d) in $CHCl_3$ yielded [2]rotaxane **45** in a 30% yield using only 1.2 equivalents of ¾-macrocycle. No [2]catenanes were isolated and the thread was fully recovered.



Scheme 7. Synthesis of a functionalized [2]rotaxane via endotopic ¾-macrocycle. Reagents and conditions: a) mono-Boc protected p-xylylenediamine, Et_3N, $CHCl_3$, 65%; b) CH_2Cl_2/TFA (9:1), then NaOH (aq) 1M, CH_2Cl_2, 95%; c) $SOCl_2$, $(COCl)_2$, reflux, quantitative; d) **39A**, **44**, Et_3N, $CHCl_3$, 30%.

The higher yield of rotaxane **45** compared to rotaxane **42** (which differ only in that the former has a macrocycle bearing an endotopic NH, and the latter does not) can be explained by the use of just chloroform in the rotaxane forming reaction, and also by the pre-organised structure of **44**, in comparison to **41**. For **44**, the endotopic nitrogen energetically favours a *syn-syn* conformation through hydrogen bonding of the pyridine nitrogen to the two amide NHs.

2.2.3.2 Characterization of [2]rotaxane 45

The ^{1}H NMR (400 MHz, 298 K) spectra of thread **4** and [2]rotaxane **45** in $CDCl_3/CD_3OD$ (9:1) (Figure 8a and 8b) shows the upfield shifting of the *E*-alkene protons of the thread (1.21 ppm). Significant is the chemical shift at very low field of the two NH protons of the macrocycle next to the endotopic nitrogen of the pyridine moiety when compared to the other two NH protons. There is a difference of nearly 2.5 ppm between the two amide sets of protons.

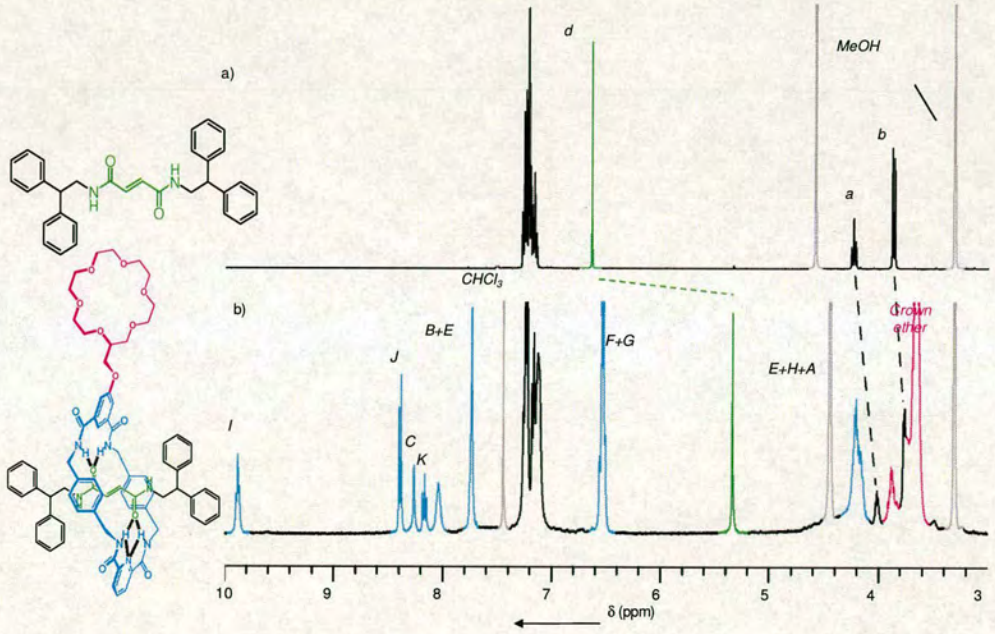
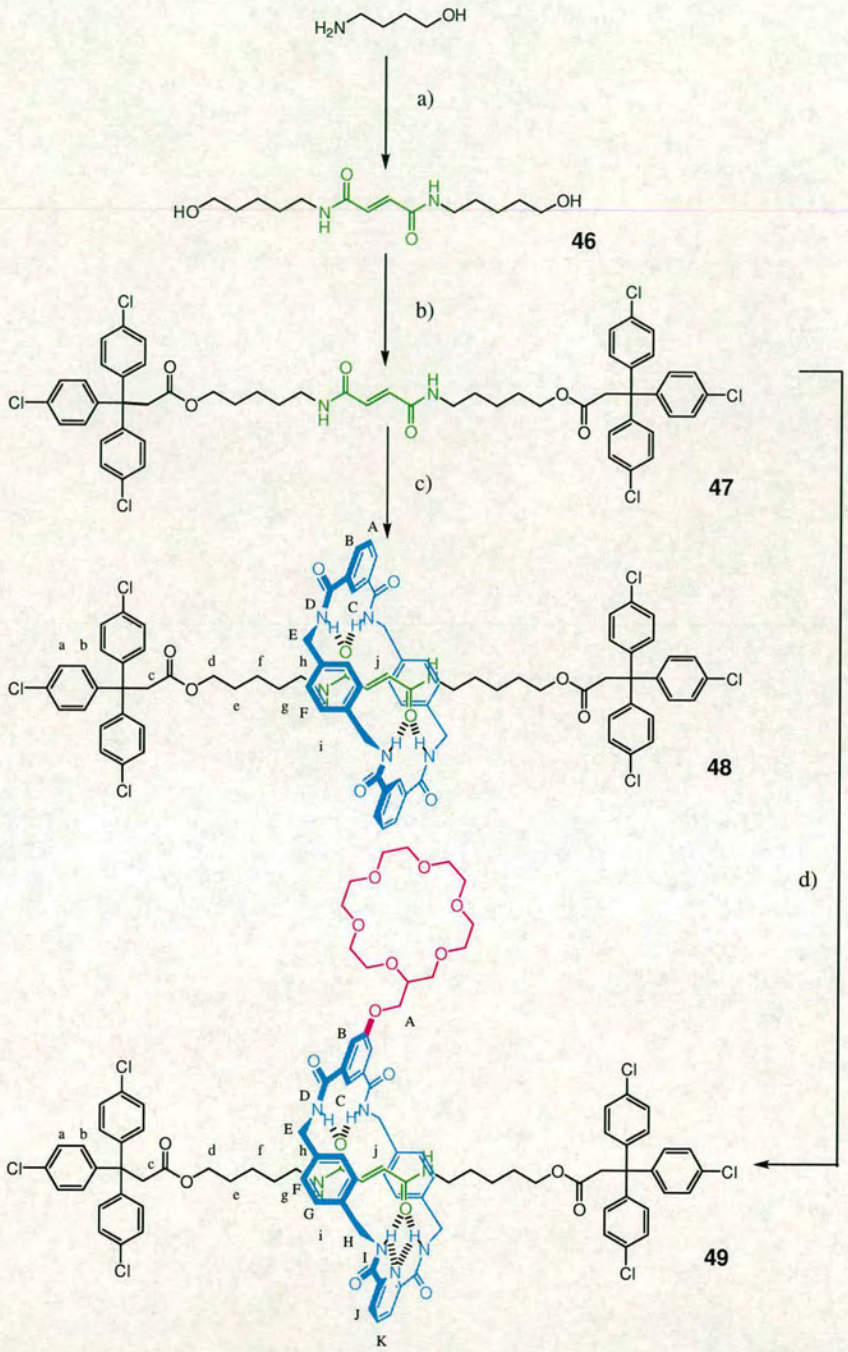


Figure 8. ^{1}H NMR [400 MHz, 298 K, $CDCl_3/CD_3OD$ (9:1)] spectra of (a) thread **4** and (b) rotaxane **45** (for hydrogen labels refer to scheme 7).

2.2.4 Synthesis of a [2]rotaxane based on a new thread with high solubility in halogenated solvents via non functionalized ¾-macrocycle

Due to the low solubility of **42** and **45** in halogenated solvents, the synthesis of a new rotaxane based on the same macrocycle with different thread components was investigated. The new thread still contained the essential fumaramide template but modified stopper functionality: being based on a tris-chlorophenyl moiety it was assumed that its solubility would increase. Scheme 5 shows the synthesis of rotaxanes **48** and **49**. 5-Amino pentanol was reacted with fumaroyl dichloride in DMF to give **46** in 70% yield (Scheme 8, step a), which was subsequently reacted with tris-chlorophenyl-propionic acid using an EDCI coupling reaction to give thread **47** in 84% yield (Scheme 8, step b). Rotaxane **48** was obtained in 95% yield through the well known five component clipping reaction adding simultaneously isopthaloyl dichloride and xylylene diamine in chloroform (Scheme 8, step c). The macrocyclization of **44** and **39A** around thread **47** in $CHCl_3$ gave [2]rotaxane **49** in 41% using 1.2 equivalents of ¾ macrocycle and substituted dichloride component (Scheme 8, step d). The absence of hydrogen bond disrupting solvents explains the higher yield of [2]rotaxane **49** compared to **45** which was subsequently improved up to 70% when an excess (8 eq) of ¾ macrocycle was used.[16] With this approach, as in the previous ones, the thread was fully recovered and a minimum amount of [2]catenanes and macrocycle was also isolated (up to 11% when 8 eq of preformed macrocycle were used).



Scheme 8. Synthesis of rotaxanes **48** and **49**. Reagents and yields: a) fumaroyl dichloride, DMF, 70%; b) 3,3,3-Tris-(4-chloro-phenyl)-propionic acid, EDCI, DMAP, CH_2Cl_2, 84%; c) isophthaloyl dichloride, xylylene diamine, Et_3N, $CHCl_3$, 95%. d) **44**, **39A**, Et_3N, $CHCl_3$, 70%.

2.2.4.2 Characterization of rotaxane 49

The ^{1}H NMR (400 MHz, 298 K) spectra of thread **47** and [2]rotaxanes **49** in $CDCl_3$ are shown in Figure 9a and 9b.

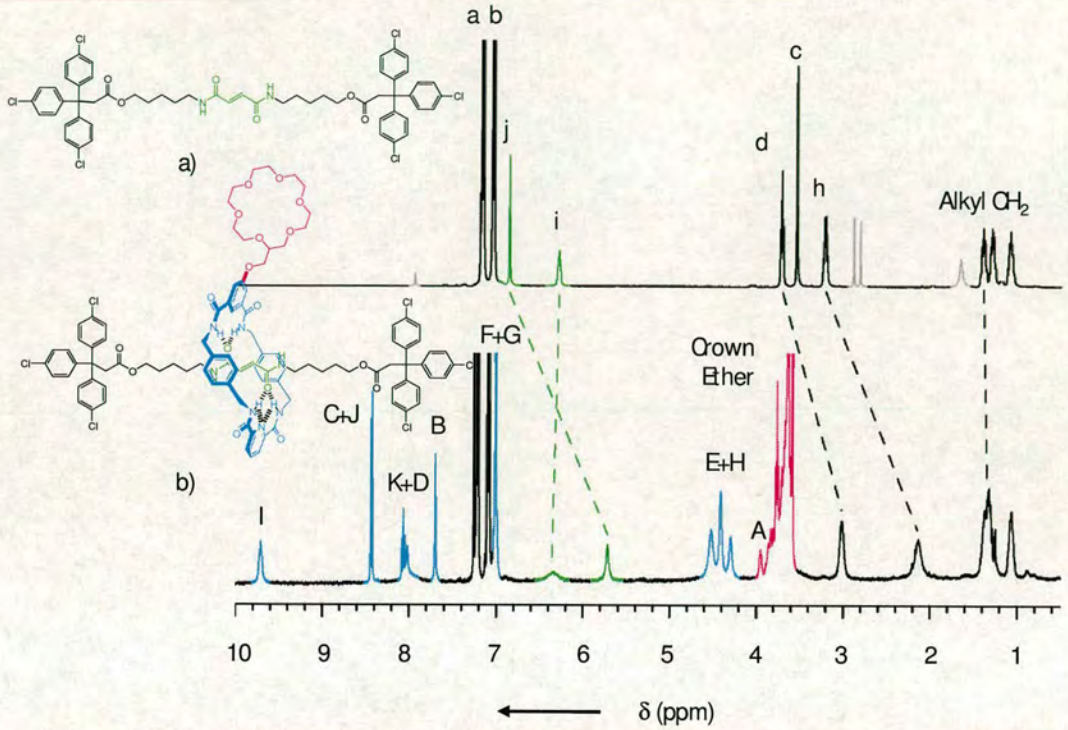


Figure 9. ^{1}H NMR [400 MHz, 298 K, $CDCl_3$] spectra (a) thread **47** and (b) rotaxane **49** (for hydrogen labels refer to scheme 8).

Unlike the previous rotaxanes reported in this chapter, the improved solubility of **49** made it possible to compare the two NMR spectra using only $CDCl_3$ as solvent. Comparison with previous spectra taken in DMSO (Figure 7) and $CHCl_3$/MeOH 9:1 (Figure 8) Figure 9 shows broadening of the rotaxane peaks: this is due to the slow rotation of the macrocycle around the thread consequent to the less polar solvent used for the experiment. The *E*-alkene H_j and methylenic H_h protons of the thread are the most influenced by the xylylene aromatic unit of the macrocycle as these protons are shifted upfield upon rotaxane formation by 0.99 ppm and 0.89 respectively. Also the H_d protons are shifted upfield by 0.6 ppm indicating that the macrocycle resides partly over

the fumaric template and partly it is hydrogen bonding the ester moiety at the other end of the thread.[17] The methylenic E and H protons of the macrocycle introduce another significant difference when compared to rotaxane **42**; the spectra show distinct sets of signals not observed in the NMR spectra of rotaxane **42** (Figure 8b).

2.2.4.3 Characterization of rotaxane 48 and 49

The comparison of the NMR spectra of thread **47**, rotaxane **48** (based on a symmetric macrocycle) and rotaxane **49** (based on a functionalised one) in the same solvents is rather interesting. For solubility reasons the NMR spectra are performed in more polar solvents. The ^{1}H NMR (400 MHz, 298K) spectra of thread **47** and rotaxanes **48** and **49** in $CDCl_3/CD_3OD$ (9:1) (Figure 10) show the upfield shifting of the E-alkene protons of the thread in both rotaxanes. The presence of the polar and H bonding disrupting solvent CD_3OD influences the positioning of the macrocycle in rotaxane **49**: when in $CDCl_3$ it sits onto the fumaramide station and the methylenic proton H_d; in presence of CD_3OD, this solvent competes for H bonding, displacing the macrocycle from the ester moiety of the thread; CD_3OD is however not sufficiently polar to displace the macrocycle from the fumaric station, where it now resides. An upfield shifting of the methylenic protons adjacent to the fumaric template H_h is observed in rotaxanes **48** and **49** confirming that the macrocycles are residing mostly over the fumaramide station. Broadening of the NMR peaks of rotaxane **49** is observed when compared to rotaxane **48**. This could be explained by the presence of the crown ether in rotaxane **49** which influences the pirouetting of the macrocycle upon interactions with other parts of the thread.

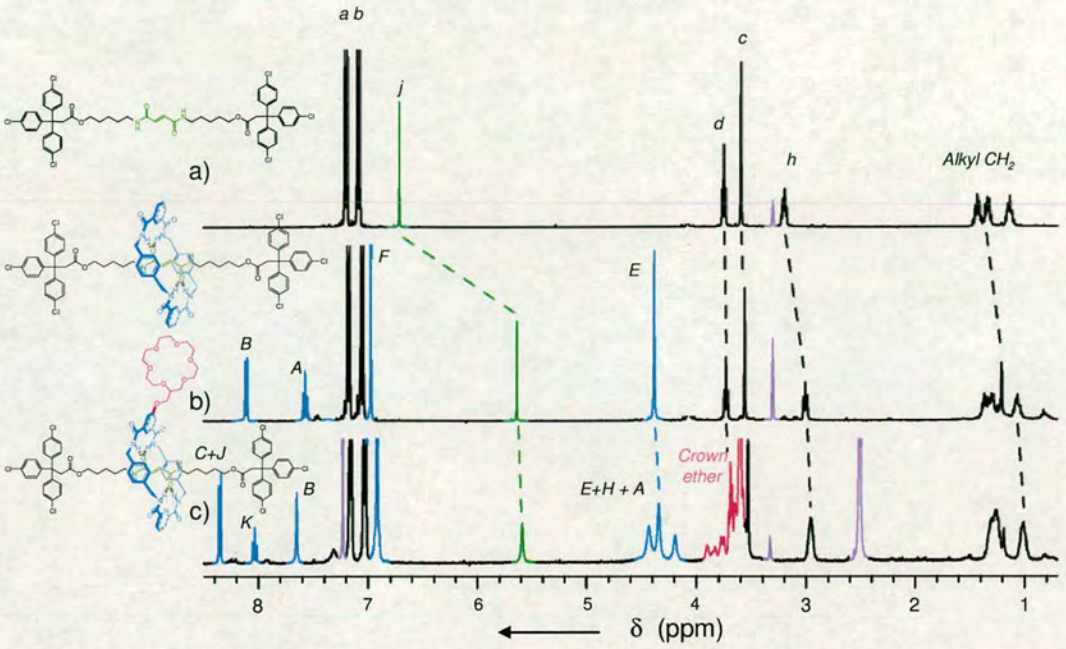
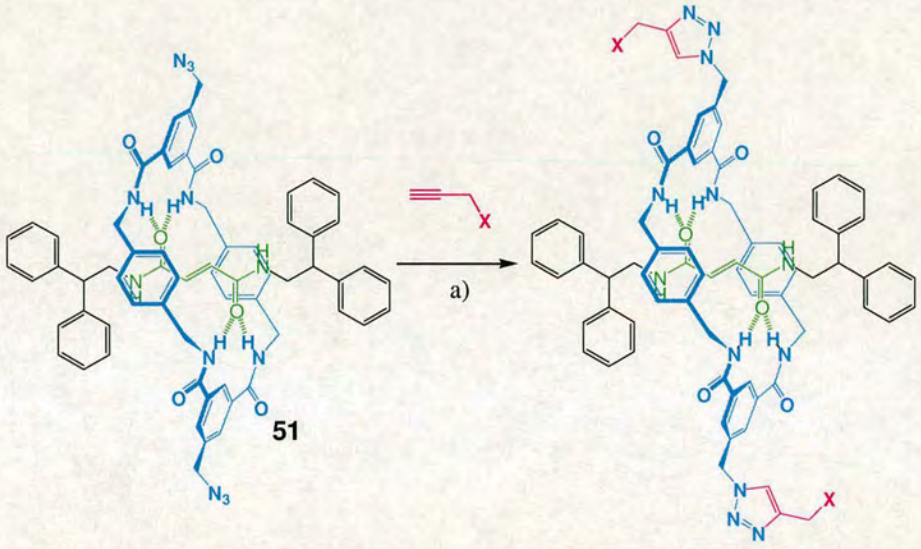


Figure 10. ^{1}H NMR [400 MHz, 298 K, $CDCl_3/CD_3OD$ 9:1] spectra (a) thread **47**, (b) rotaxane **48** and (c) rotaxane **49** (for hydrogen labels refer to scheme 8).

2.3 Post-modification of a [2]rotaxanes via the "click" reaction

In the previous paragraphs three different routes to the formation of rotaxanes with mono-functionalized macrocycles were shown. They are different approaches to solving the problem of synthesizing mono-functionalized macrocycles. The first two strategies involve a functionalized ¾-macrocycle; in the third method a non-functionalized ¾-macrocycle is used and the functionalization is provided by the isophthalic component. While the yields are comparable the latter proved to be the best approach thanks to the solubility of the ¾-macrocycle. Another advantage is that several rotaxanes can be synthesized through simple straightforward variations of the single isophthalic component.

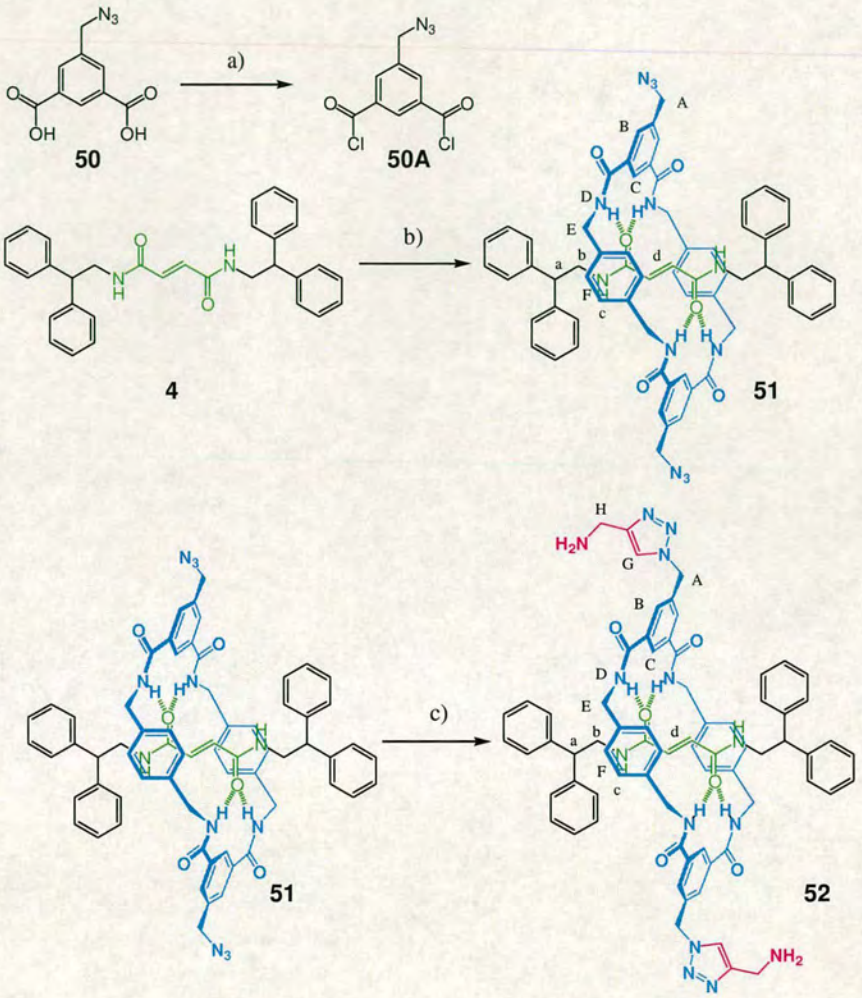
A more modular approach to rotaxanes with different mono-functionalized macrocycles is via a post-modification strategy i.e. using a single mono-functionalized parent rotaxane that can be readily modified at will. In this paragraph the post-di-modification of the macrocycle is described (Scheme 9): families of difunctionalized macrocycles can be prepared by exploiting the rediscovered Sharpless[18-20] "click" reaction a Cu(I) catalysed 1,3-cycloaddition reaction between organic azides and terminal alkynes. Previous work from the Leigh group had shown the click reaction to be a powerful tool for the synthesis of [2]rotaxanes.[21]



Scheme 9. Post-modification of the macrocycle via "Click" reaction. a) $Cu(I)(CH_3CN)_4PF_6$, alkyne bearing a functionalized group X, CH_3OH, $CHCl_3$.

Compound **50** was prepared from benzenedicarboxylic acid as reported by Toone and co-workers.[22] 5-Azidomethyl-isophthaloyl dichloride, **50A** (Scheme 10, step a), was obtained in quantitative yield from refluxing a solution of **50** with thionyl chloride and oxalyl chloride. Subsequent treatment of thread **4** with 6 equivalents of the dichloride **50A** and xylylene diamine in chloroform and Et_3N yielded [2]rotaxane **51** in a good 70% yield (Scheme 10, step b). Propargyl amine was then "clicked" to rotaxane **51** using a

commercially available source of copper (I) in $CHCl_3$ and methanol to furnish [2]rotaxane **52** in high yield (Scheme 10, step c).



Scheme 10. Post-modification of the macrocycle via "Click" reaction. Reagents and yield: a) $SOCl_2$, $(COCl)_2$, reflux, quantitative; b) **4**, **50A**, xylylene diamine, Et_3N, $CHCl_3$, 70%; c) $Cu(I)(CH_3CN)_4PF_6$, propargyl amine, CH_3OH, $CHCl_3$, 95%.

2.3.1 Characterization of rotaxanes 50 and 51

The ^1H NMR (400 MHz, 298 K) spectra of thread **4** and [2]rotaxanes **51** and **52** in DMSO- d_6 (Figure 12a, 12b and 12c) show the upfield shifting of the *E*-alkene protons

of the thread (0.99 ppm). After formation of the triazole ring there are no major changes in the product spectrum except the methylenic protons adjacent to the triazole ring H_A which are shifted downfield by 1.08 ppm.

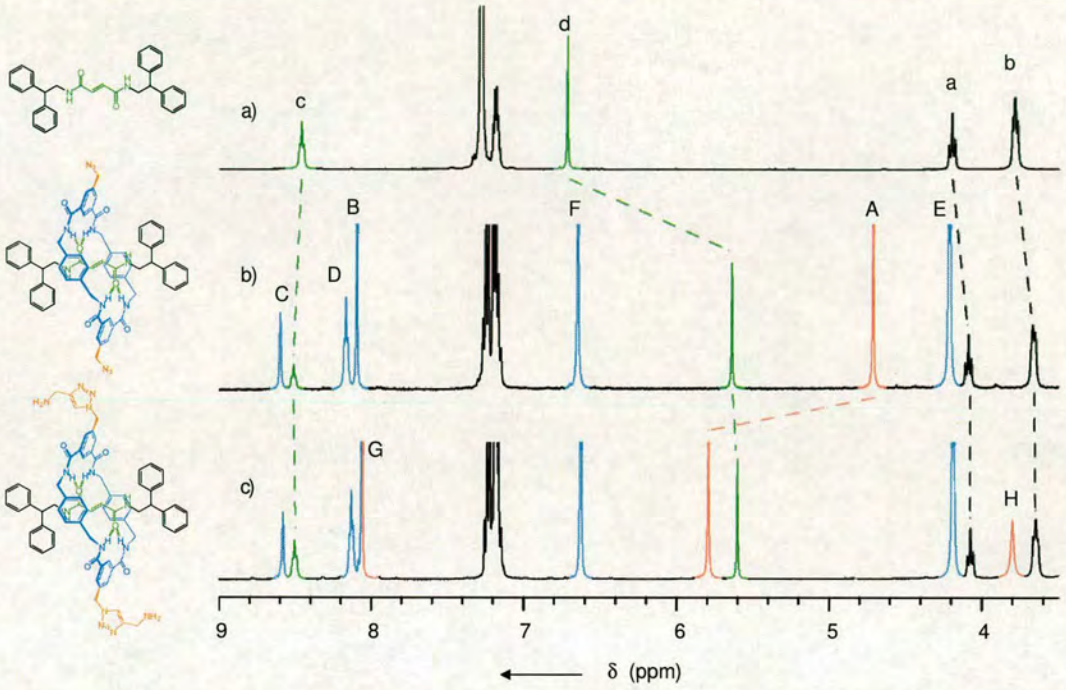


Figure 11. ^{1}H NMR [400 MHz, 298 K, DMSO- d_6] spectra of (a) thread **4**, (b) rotaxane **51** and (c) rotaxane **52** (for hydrogen labels refer to scheme 10).

Two solid state structures of **51** from crystals grown from DMF/Et_2O (Figure 12), a hydrogen bonding disruptive solvent, and MeOH/CH_2Cl_2 (Figure 13a and 13b), poorer hydrogen-bonding disrupting solvent, were obtained. In figure 16 the macrocycle adopts a chair-like conformation with its four amide hydrogens forming double bifurcated, intramolecular hydrogen bonds orthogonal to the lone pairs of the fumaramide carbonyl groups. Two intermolecular hydrogen bonds are formed between the two fumaramide hydrogens of the thread and the oxygens of two water molecules. Another 4 intermolecular hydrogen bonds are revealed by the crystal packing and are formed

between two carbonyl units belonging to two adjacent macrocycles and the four protons from the two water molecules.

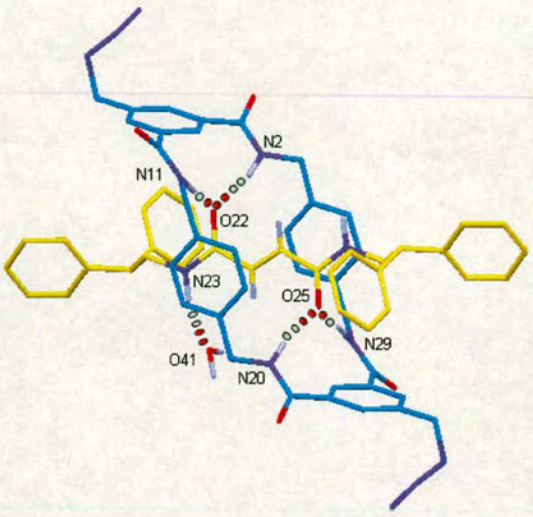


Figure 12. X-ray crystal structures of the fumaramide-based [2]rotaxane **51** crystallized from DMF/Et_2O. The carbon atoms of the macrocycles are shown in blue, carbon atoms of the threads in yellow, oxygen atoms in red and nitrogen atoms in dark blue. The amide and alkene hydrogen atoms are shown in white while all others are removed for clarity. Intramolecular hydrogen bond distances (Å) are the following: (a) O22-HN2/O25-HN20 = 2.05, O22-HN11/O25-HN29 = 1.91, O41-HN23 = 1.90.

When the rotaxane was crystallized in MeOH/CH_2Cl_2, as opposed to DMF, the number of intramolecular hydrogen bonds decreases from the maximum four possible to two and a different crystal lattice is observed (Figure 13a). The loss in the number of intramolecular hydrogen bonds is compensated for by the formation of two sets of bifurcated, intermolecular hydrogen bonds with adjacent rotaxanes as revealed by the crystal packing (Figure 13b).

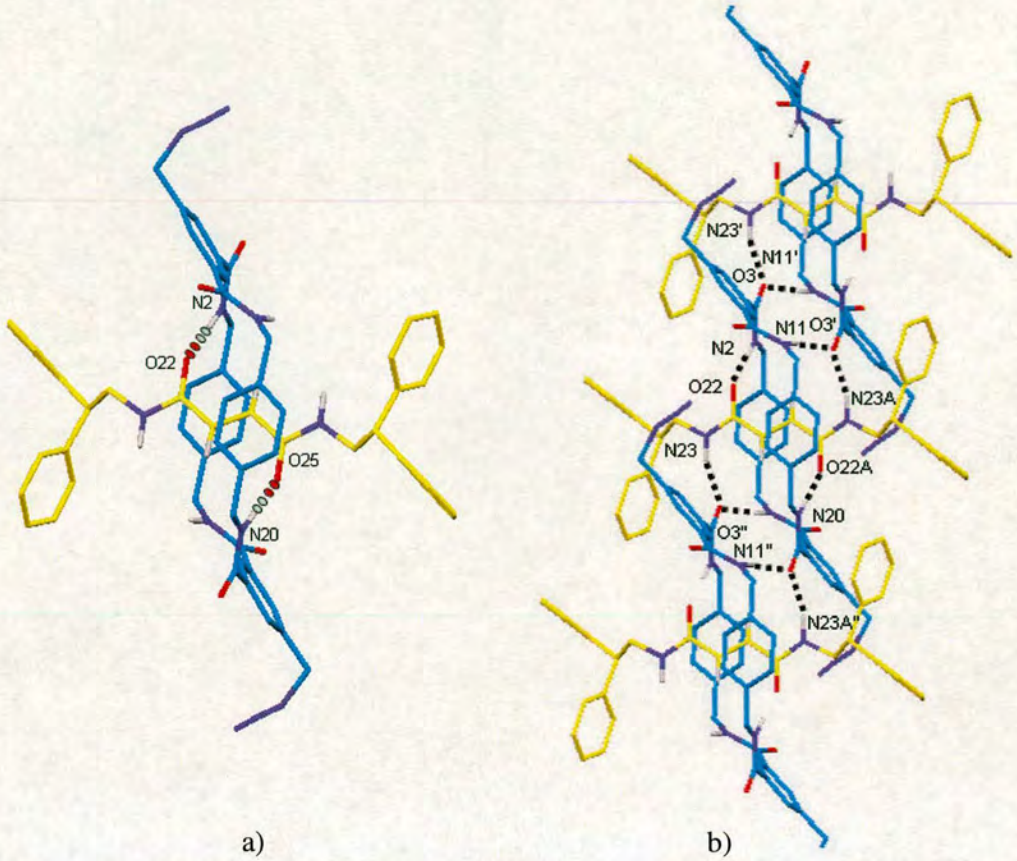


Figure 13. *X*-ray crystal structures of the fumaramide-based [2]rotaxanes **51** crystallised from CH_3OH/CH_2Cl_2 (a) as single molecule and (b) showing the crystal packing. The carbon atoms of the macrocycles are shown in blue, carbon atoms of the threads in yellow, oxygen atoms in red and nitrogen atoms in dark blue. The amide and alkene hydrogen atoms are shown in white while all others are removed for clarity. Intramolecular hydrogen bond distances (Å): O40-HN2/O43-HN20 = 1.99. Intermolecular hydrogen bond distances (Å): O22-HN2/O25-HN20 = 1.81, O3'-HN11 = 2.11, O3''-HN23/O3'-HN23A = 1.99.

A solid state structure of **52** was obtained using crystals grown from DMF/Et_2O (Figure 14). The macrocycle adopts a perfect chair-like conformation with its four amide hydrogens forming double bifurcated, intramolecular hydrogen bonds orthogonal to the lone pairs of the fumaramide carbonyl groups. Two more hydrogen bonds are formed between two carbonyl groups of the solvent molecules (DMF) and the fumaramide NH hydrogens of the thread.

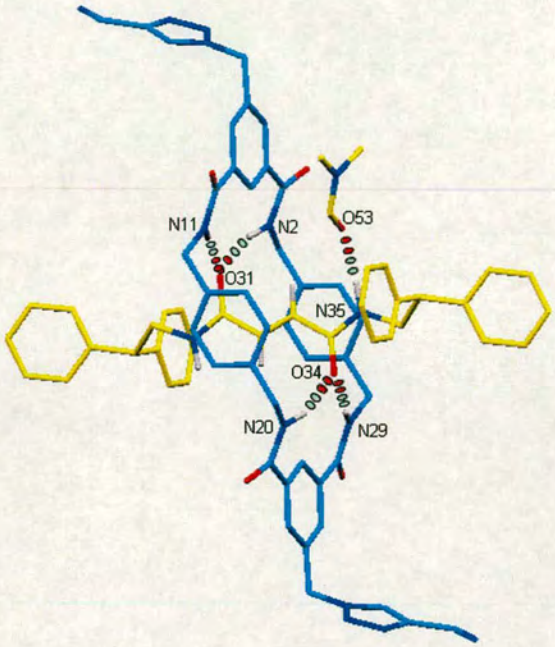


Figure 14. *X*-ray crystal structures of the fumaramide-based [2]rotaxane **52** crystallised from DMF/Et_2O. The carbon atoms of the macrocycles are shown in blue, carbon atoms of the threads in yellow, oxygen atoms in red and nitrogen atoms in dark blue. The amide and alkene hydrogen atoms are shown in white; all others are removed for clarity. Intramolecular hydrogen bond distances (Å): (a) O31-HN2/O34-HN20 = 2.10, O31-HN11/O34-HN29 = 2.11, O53-HN35 = 1.83.

2.4 Conclusions

In this chapter we have shown that it is possible to make a variety of mono- and di-functionalized [2]rotaxanes and macrocyclic architectures.

We have illustrated that our investigation on new synthetic routes to mono-functionalized macrocycles has lead to a major improvement over the previously existing poor yielding statistical approach.

We have discovered that higher yields are obtained when a ¾ macrocycle with an endotopic nitrogen is used in the presence of non-disrupting hydrogen bonding solvents.

Finally, we have proven that a Sharpless "click" reaction can be used to functionalize the macrocycle leading to improved yields and simpler and quicker laboratory synthetic procedure. This strategy allows the generation of families of functionalized [2]rotaxanes, created from a single original rotaxane derivatised via post-functionalization of the macrocycle.

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[16] A high amount of ¾-macrocycle components can only be convenient when small single station threads are used. In systems containing more than one station and longer threads there's the possibility of formation of [3]rotaxane since the thread,

being quite long, means that there is ample space for the interlocked macrocycle to move away from the fumaric template, allowing another macrocycle to form around this site.

[17] Shifting of the methylenic protons next to the ester bond could be due to the folding of the thread induced by the macrocycle.

[18] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004-2021.

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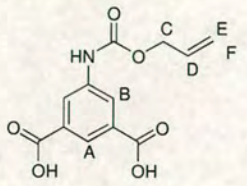
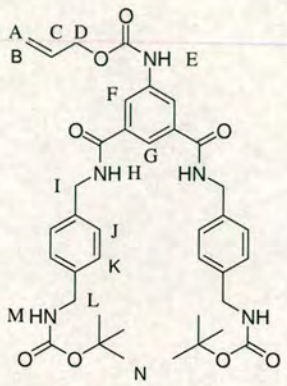
Experimental part

Preparation of 5-Allyloxycarbonylamino-isophthalic acid (32).



To a solution of 5-amino isophthalic acid (5.00 g, 27.6 mmol) in NaOH 1M (150 mL) was added a solution of allyl chloroformate (4.00 g, 33.1 mmol) in dioxane (150 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred vigorously for 12 h. HCl 2M was added until pH = 2 and the dioxane was removed under reduced pressure. The aqueous layer was extracted with EtOAc (2 × 100 mL), washed with brine (sat., 200 mL), dried over Na_2SO_4, filtered and evaporated under vacuum. The crude product was triturated with *n*-hexane. The resulting precipitate was collected by filtration, washed with diethyl ether and dried under reduced pressure to afford **1** as a colorless solid (7.10 g, 97%); mp: 155 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 12.91 [s, 2H, COOH], 10.13 [s, 1H, NH], 8.31 [d, 2H, J = 1.2 Hz, Ar-H_B], 8.10 [t, 1H, J = 1.4 Hz, Ar-H_A], 5.98 [m, 1H, CH_D], 5.37 [dd, 1H, J = 1.6 Hz, J = 15.6 Hz CH_E or CH_F], 5.25 [dd, 1H, J = 1.4 Hz, J = 11.8 Hz, CH_E or CH_F], 4.64 [d, 2H, J = 5.5 Hz, CH_C]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 166.5, 153.2, 140.2, 133.1, 132.8, 123.8, 122.6, 117.8, 65.0; FABMS: m/z = 266 $[M+H]^+$; HRMS : m/z = 266.0662 $[M+H]^+$ (anal. calcd. for $C_{12}H_{12}NO_6^+$: m/z = 266.0664).

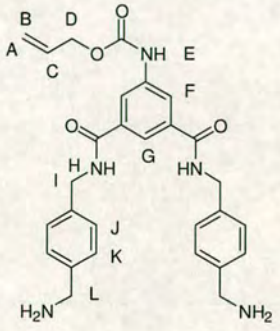
Preparation of {3,5-Bis-[4-(tert-butoxycarbonylamino-methyl) benzylcarbamoyl] -phenyl}-carbamic acid allyl ester (33).



Carboxylic acid **32** (1.57 g, 5.93 mmol) was dissolved in DMF (50 mL) and the solution was cooled to 0 °C. EDCI·HCl (4.0 g, 20.90 mmol), 1-hydroxybenzotriazole hydrate (2.82 g, 20.90 mmol) and Et_3N (4.6 g, 32.73 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 30 min. A solution of mono-Boc protected p-xylylenediamine in DMF (Sigma-Aldrich) (20 mL) (3.08 g, 20.9 mmol) was added to the activated acid. The reaction mixture was stirred for 36 h, concentrated under reduced pressure and then CH_2Cl_2 was added (200 mL). The organic layer was washed with 1M HCl (50 mL), with $NaHCO_3$ (sat. aq., 50 mL). The organic layer was further washed with brine (sat., 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (gradient elution: CH_2Cl_2 / EtOH 97:3 then CH_2Cl_2 / EtOAc 6:4) to furnish compound **2** as a colourless solid (3.07 g, 75%); mp: 115 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 9.97 [s, 1H, NH_E], 8.95 [t, 2H, J = 5.8 Hz, NH_H], 8.06 [s, 2H, Ar-H_F], 7.91 [s, 1H, Ar-H_G], 7.82 [t, 1H, J = 6.0 Hz, NH_M], 7.21 [d, 4H, J = 7.9 Hz, Ar-H_J or Ar-H_K], 7.12 [d, 4H, J = 7.9 Hz, Ar-H_J or Ar-H_K], 5.92 [m, 1H, CH_C], 5.31 [d, 1H, J = 15.8 H, CH_A or CH_B], 5.18 [d, 2H, J = 10.5 Hz, CH_A or CH_B], 4.57 [d, 2H, J = 5.5 Hz, CH_D], 4.40 [d, 4H, J = 5.3 Hz, CH_I], 4.06 [d, 4H, J = 5.9 Hz, CH_L], 1.35 [s, 18H, CH_N]; ^{13}C NMR (400 MHz, $CDCl_3$/ d_6-DMSO 1 :1): δ = 165.9, 157.2, 155.7, 153.1, 139.3, 138.6, 137.6, 135.1, 132.7, 127.3,

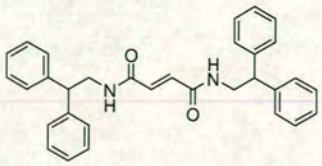
126.7, 120.0, 119.5, 117.4, 80.0, 64.7, 43.2, 42.6, 28.1; FABMS: *m/z* = 702 $[M+H]^+$; HRMS : *m/z* = 702.3513 $[M+H]^+$ (anal. calcd. for $C_{38}H_{48}N_5O_8^+$: *m/z* = 702.3502).

Preparation of [3,5-Bis-(4-aminomethyl-benzylcarbamoyl)-phenyl]-carbamic acid allyl ester (34).



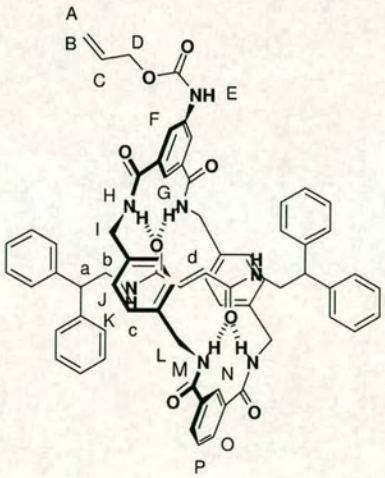
A solution of **33** (1.0 g, 1.42 mmol) in CH_2Cl_2 / TFA 9:1 (40 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure to remove TFA and then CH_2Cl_2 (300 mL) was added. The organic layer was washed with $NaHCO_3$ (sat. aq., 50 mL) dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was triturated twice with diethyl ether to give the product as colourless solid. (483 mg, 68%); mp = 170 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 10.05 [s, 1H, NH_E], 9.15 [t, 2H, *J* = 6.0 Hz, NH_H], 8.19 [s, 4H, NH_2], 8.07 [s, 2H, *J* = 1.1 Hz, Ar-H_F], 7.97 [s, 1H, Ar-H_G], 7.40 [d, 4H, J = 8.2 Hz, Ar-H_J and Ar-H_K], 7.36 [d, 4H, J = 8.3 Hz, Ar-H_J and Ar-H_K], 5.97 [m, 1H, CH_C], 5.38 [d, 1H, *J* = 1.6 Hz, *J* = 15.6 H, CH_A or CH_B], 5.24 [d, 1H, *J* = 1.5 Hz, *J* = 9.0 Hz, CH_A or CH_B], 4.62 [d, 2H, *J* = 5.4 Hz, CH_D], 4.46 [d, 4H, *J* = 5.8 Hz, CH_I], 3.99 [d, 4H, *J* = 5.9 Hz, CH_L]; ^{13}C NMR (400 MHz, DMSO-d_6): δ = 165.9, 157.2, 157.1, 153.2, 140.0, 139.3, 135.3, 133.0, 132.4, 128.8, 127.5, 117.7, 64.8, 42.4, 41.9. FABMS: *m/z* = $[M+H]^+$; HRMS : *m/z* = 502.2455 $[M+H]^+$ (anal. calcd. for $C_{28}H_{32}N_5O_4^+$: *m/z* = 502.2454).

Preparation of *N,N'–bis* (2,2–diphenyl-ethyl)- (*E*)-butendiamide (4).



Thread **4** was prepared as reported by Leigh and coworkers.[1]

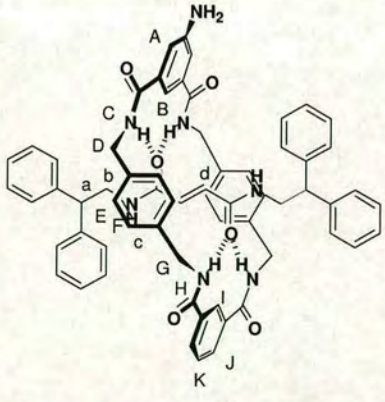
Preparation of [2](1,9,16,22-Tetraaza-2,8,17,21-tetraoxo-5-carbamic acid allyl ester-3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-(*N,N'–bis*(2,2–diphenyl-ethyl)-(*E*)-butendiamide)-rotaxane (35).



Thread **4** (300 mg, 0.63 mmol) and **34** (631 mg, 1.26 mmol) and Et_3N (1.78 mL, 12.6 mmol) in anhydrous $CHCl_3/CH_3CN$/DMF 90:8:2 (200 mL) were stirred vigorously whilst solution of isophthaloyl dichloride (291 mg, 1.38 mmol) in anhydrous $CHCl_3$ (20 mL) was simultaneously added over a period of 2 h using motor-driven syringe pumps. After 24 h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography (eluant: CH_2Cl_2 / MeOH, 97:3) to furnish rotaxane 3**5** as white solid. (31%); mp = 250 °C (decomp); ^1H

NMR (400 MHz, d_6-DMSO): δ = 10.14 [s, 1H, NH_E], 8.60 [s, 1H, Ar-H_N], 8.52 [t, 2H, J = 5.3 Hz, NH_c], 8.47 [s, 1H, Ar-H_G], 8.24-8.00 [m, 8H, NH_H NH_M, Ar-H_F and Ar-H_O], 7.70 [t, 1H, J = 7.7 Hz, Ar-H_P], 7.33-7.10 [m, 20H, Ar-H_{thread}], 6.63 [s, 8H, Ar-H_J and Ar-H_K], 6.02 [m, 1H, CH_C], 5.56 [s, 2H, CH_d], 5.40 [dd, 1H, J = 1.6 Hz, J = 15.6 Hz, CH_A or CH_B], 5.27 [dd, 1H, J = 1.4 Hz, J = 9.0 Hz, CH_A or CH_B], 4.67 [d, 2H, J = 5.4 Hz, CH_D], 4.19 [s, 8H, CH_I and CH_L], 4.08 [t, 2H, J = 7.6 Hz, CH_a], 3.65 [dd, 4H, J = 6.5 Hz, CH_b]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 173.7, 166.5, 165.7, 165.6, 165.2, 157.3, 153.2, 142.5, 136.3, 136.2, 135.0, 134.2, 133.4, 133.1, 131.1, 130.7, 129.9, 129.2, 129.1, 128.5, 127.7, 126.5, 117.7, 64.8, 49.8, 43.6, 43.3, 43.1; FABMS: m/z = 1106 $[M+H]^+$; HRMS : m/z = 1106.4815 $[M+H]^+$ (anal. calcd. for $C_{68}H_{64}N_7O_8^+$: m/z = 1106.4816).

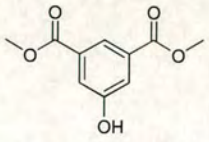
Preparation of [2](1,9,16,22-Tetraaza-2,8,17,21-tetraoxo-5-amino-3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-(*N,N'–bis*(2,2–diphenyl-ethyl)-(*E*)-butendiamide)-rotaxane (36).



A solution of rotaxane **35** (390 mg, 0.35 mmol) in THF (15 mL) and dimedone (246 mg, 2.47 mmol) was stirred with tetrakis(triphenylphosphane) palladium (0) (5 mg, 0.004 mmol) for 24 h at rt. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (gradient elution: CH_2Cl_2 / EtOH 97:3 then CH_2Cl_2 / EtOAc 6:4) to furnish compound **36** as a colorless solid (318 mg, 89%);

Mp = 250 °C (decomp); ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.61 [s, 1H, Ar-H_I], 8.54 [t, 2H, *J* = 5.6 Hz, NH_c], 8.14 [t, 2H, J = 5.0 Hz, NH_C or NH_H], 8.09 [d, 2H, *J* = 1.4 Hz, Ar-H_A], 8.07 [d, 2H, *J* = 1.4 Hz, Ar-H_J], 7.98 [t, 2H, *J* = 4.8 Hz, NH_C or NH_H], 7.76 [s, 1H, Ar-H_B], 7.71 [t, 1H, *J* = 7.7 Hz, Ar-H_K], 7.33-7.12 [m, 20H, Ar-H_{thread}], 6.61 [s, 8H, Ar-H_E and Ar-H_F], 5.60 [s, 2H, CH_d], 4.17 [dd, 8H, *J* = 4.9 Hz, *J* = 6.6 Hz, CH_D and CH_G], 4.11 [t, 2H, *J* = 7.7 Hz, CH_a], 3.65 [dd, 4H, *J* = 6.4 Hz, CH_b]; ^{13}C NMR (400 MHz, d_7-DMF): δ = 166.4, 166.2, 165.7, 165.1,159.4, 149.8, 142.9, 137.3, 137.2, 137.0, 135.6, 135.3, 134.4, 130.9, 129.7, 129.0, 128.6, 128.2, 128.0, 124.9, 124.3, 50.5, 44.0, 43.6; FABMS: *m/z* = 1022 $[M+H]^+$; HRMS : *m/z* = 1022.4606 $[M+H]^+$ (anal. calcd. for $C_{64}H_{60}N_7O_6^+$: *m/z* = 1022.4605).

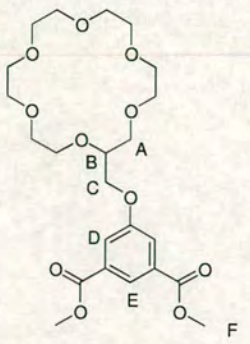
Preparation of 5-hydroxyisophthalic acid dimethyl ester (37).



A stirred solution of 5-hydroxy isophthalic acid (10.0 g, 48 mmol) and concentrated sulfuric acid (1.4 mL) in methanol (100 mL) was refluxed for 72 hours and then cooled to afford a colorless precipitate. The precipitate was recovered by filtration and washed with water until neutral before being dried under reduced pressure to afford the desired ester **1** as colorless solid (9.17 g, 91%).

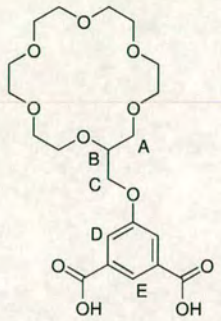
This compound was prepared as described in our group and showed identical spectroscopic data according to literature.[2]

Preparation of 5-(1,4,7,10,13,16hexaoxa-cyclooctadec-2-ylmethoxy)-isophthalic acid dimethyl ester (38).



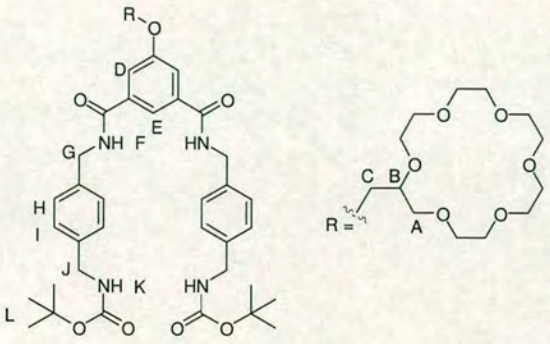
A mixture of **37** (820 mg, 3.87 mmol) 18-crown-6 methanol (950 mg, 3.23 mmol) and triphenylphosphine (1.27 g, 4.84 mmol) in THF (20 mL) was cooled to 0°C. Then diisopropylazadicarboxylate (DIAD) (1.0 mL, 4.84 mmol) was added dropwise and the reaction was stirred overnight at room temperature under nitrogen atmosphere. THF was removed under reduced pressure and the product was isolated by flash chromatography (gradient elution: CH_2Cl_2 / MeOH 97:3 then CH_2Cl_2 / MeOH 9:1) to furnish compound **38** as a colourless oil (1.33 g, 81%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27 [t, 1H, J = 1.4 Hz, Ar-H_E], 7.77 [d, 2H, J= 1.4 Hz, Ar-H_D], 4.17 [m, 2H, CH_C], 4.00 [m, 1H, CH_B], 3.93 [s, 6H, CH_F], 3.87 [m, 2H, CH_A], 3.80-3.55 [m, 20H, Crown (O-CH_2-)]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 166.1, 157.9, 131.7, 123.1, 120.0, 71.1, 71.1, 70.8, 70.7, 52.4; FABMS: *m/z* = 487 $[M+H]^+$; HRMS : *m/z* = 487.2172 $[M+H]^+$ (anal. calcd. for $C_{23}H_{35}O_{11}^+$: *m/z* = 487.2179).

Preparation of 5-(1,4,7,10,13,16hexaoxa-cyclooctadec-2-ylmethoxy)-isophthalic acid (39).



To a stirred solution of **38** (625 mg, 1.28 mmol), in THF (10 mL) and H_2O (1 mL), was added dropwise a solution of NaOH (134 mg, 3.33 mmol) in H_2O (1.5 mL). After 16 h the solution was reduced in volume and triturated several times with HCl 1M in dioxane to obtain a colourless powder. (600 mg, 96%); mp = 123-129 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.07 [t, 1H, J = 1.2 Hz, Ar-H_E], 7.66 [d, 2H, J= 1.3 Hz, Ar-H_D], 4.18 [m, 2H, CH_C], 3.87 [q, 1H, J = 4.5 Hz, CH_B], 3.80-3.38 [m, 22H, CH_A and Crown (O-CH_2-)]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 166.0, 158.3, 132.3, 122.0, 118.9, 69.7, 69.6, 69.4, 69.2, 69.1, 68.3, 67.7, 48.2; FABMS: *m/z* = 481 $[M+Na]^+$; HRMS : *m/z* = 481.1689 $[M+Na]^+$ (anal. calcd. for $C_{21}H_{30}NaO_{11}^+$: *m/z* = 481.1685).

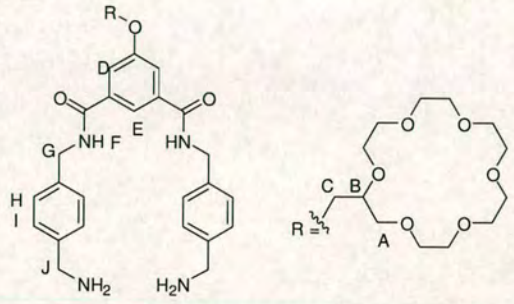
Preparation of (4-{[3-[4-(tert-Butoxycarbonylamino-methyl)-benzylcarbamoyl]-5-(1,4,7,10,13,16-hexaoxa-cyclooctadec-2-ylmethoxy)-benzoylamino]-methyl}-benzyl)-carbamic acid tert-butyl ester (40).



Carboxylic acid **39** (618 mg, 1.35 mmol) was dissolved in DMF (15 mL) and the solution was cooled to 0 °C. EDCI·HCl (1.24 g, 6.50 mmol), HOBt (0.55 g, 4.05 mmol) and Et_3N (0.93 g, 9.18 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 30 min. A solution of mono-Boc protected p-xylylenediamine in DMF (10 mL) (0.95 g, 4.05 mmol) was added to the activated acid. The reaction mixture was stirred for 36 h, concentrated under reduced pressure and then CH_2Cl_2 was added (300 mL). The organic layer was washed only once with 1M HCl (20 mL) then the organic layer was concentrated under reduced pressure. The crude material was purified by flash chromatography [gradient elution: CH_2Cl_2 / MeOH 98:2 and 0.2% of NH_3(aq) then CH_2Cl_2 / MeOH 90:10 and 1% NH_3(aq)] to furnish compound **40** as a colorless solid (0.76 g, 64%); mp = 102-104 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84 [s, 1H, Ar-H_E], 7.47 [d, 2H, J= 1.2 Hz, Ar-H_D], 7.29-7.07 [m, 10H, NH_F and Ar-H_H and Ar-H_I], 5.03 [s, 2H, NH_K], 4.49 [d, 4H, J = 4.8 Hz, CH_G], 4.21 [d, 4H, J = 5.9Hz, CH_J], 4.17 [m, 2H, CH_C], 3.91 [q, 1H, J = 4.8 Hz, CH_B], 3.87-3.48 [m, 22H, CH_A and Crown (O-CH_2-)], 1.43 [s, 18H, CH_L]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 166.3, 159.3, 156.1, 138.3, 137.1, 135.62, 128.2, 127.6, 116.7, 79.5, 71.0, 70.8, 70.6, 70.5, 70.4, 70.3, 70.0,

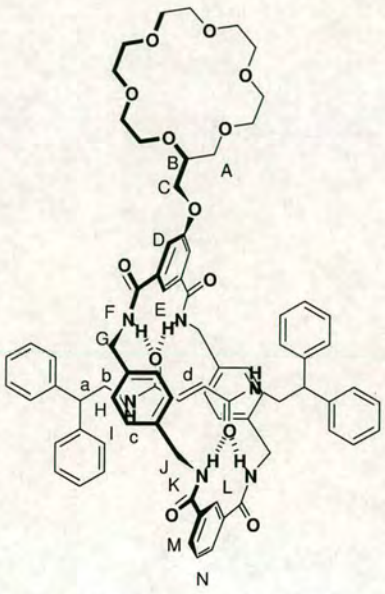
68.4, 53.4, 44.2, 43.8, 28.4; FABMS: *m/z* = 917 $[M+Na]^+$; HRMS : *m/z* = 917.4535 $[M+Na]^+$ (anal. calcd. for $C_{47}H_{66}N_4NaO_{13}^+$: *m/z* = 917.4524).

Preparation of N,N'-Bis-(4-aminomethyl-benzyl)-5 (1,4,7,10,13,16 hexaoxa-cyclooctadec-2-ylmethoxy)-isophthalamide (41).



A solution of **40** (1.05 g, 1.17 mmol) in CH_2Cl_2 / TFA 9:1 (40 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure to remove TFA and then CH_2Cl_2 (100 mL) and diethylaminomethyl-polystyrene resin [1.8 g, 5.83 mmol] were added. The organic layer was stirred for 18h then the suspension was filtered and the solvent was removed under reduced pressure. The crude material was triturated twice with diethyl ether to give the product as colourless white solid (657 mg, 81%); mp = 132-134 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 9.19 [t, 2H, J = 5.9 Hz, NH_F], 7.99 [s, 1H, Ar-H_E], 7.60 [d, 2H, Ar-H_D], 7.42-7.26 [m, 8H, Ar-H_H and Ar-H_I], 6.72 [s, 4H, NH_2], 4.53 [d, 4H, *J* = 5.7 Hz, CH_G], 4.20-4.06 [m, 2H, CH_C], 3.90 [s, 4H, CH_J], 3.71 [q, 1H, J = 4.4 Hz, CH_B], 3.65-3.46 [m, 22H, CH_A and Crown (O-CH_2-)]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 165.8, 157.7, 139.6, 136.2, 136.8, 128.7, 127.8, 122.0, 116.2, 70.6, 70.5, 70.2, 69.4, 55.5, 43.5, 42.8; FABMS: *m/z* = 695 $[M+H]^+$; HRMS : *m/z* = 695.3654 $[M+H]^+$ (anal. calcd. for $C_{37}H_{51}N_4O_9^+$: *m/z* = 695.3656).

Preparation of [2](1,9,16,22-Tetraaza-2,8,17,21-tetraoxo-5-(1,4,7,10,13,16hexaoxa-Cyclooctadec-2-ylmethoxy)- -3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-(*N,N'–bis*(2,2–diphenyl-ethyl)-(*E*)-butendiamide)-rotaxane (42).

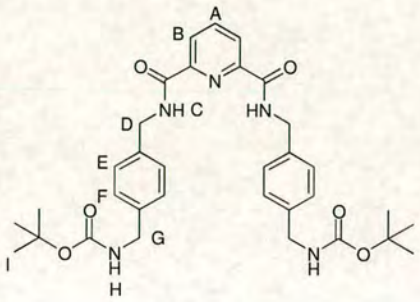


N

Thread **4** (193 mg, 0.407 mmol) and compound **41** (763 mg, 1.10 mmol) and Et_3N (1.15 mL, 8.14 mmol) in anhydrous $CHCl_3$ / CH_3CN / DMF 85:10:5 (150 mL) were stirred vigorously whilst solution of isophthaloyl dichloride (223 mg, 1.10 mmol) in anhydrous $CHCl_3$ (20 mL) was simultaneously added over a period of 2 h using motor-driven syringe pumps. After 24 h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography [gradient elution: CH_2Cl_2 / MeOH 95:5 and 0.1% of NH_3(aq) then CH_2Cl_2 / MeOH 90:10 and 1% NH_3(aq)] to furnish compound **11** as a white solid (55 mg, 10%); mp = 250 °C (decomp); ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.60 [s, 1H, Ar-H_L], 8.51 [t, 2H, J = 5.1 Hz, NH_c], 8.21 [s, 1H, Ar-H_E], 8.15 [t, 4H, J = 4.6 Hz, NH_F and NH_K], 8.08 [d, 2H, J = 7.7Hz, Ar-H_M], 7.70 [t, 1H, J = 7.8 Hz, Ar-H_N], 7.59 [s, 2H, Ar-H_D], 7.31-7.12 [m, 20H, Ar-H_{Thread}], 6.64 [s, 8H, CH_H and CH_I], 5.63 [s, 2H, CH_d], 4.45 [m, 2H, CH_C], 4.20 [s, 4H, CH_G or CH_J], 4.18 [s, 4H, CH_G or CH_J], 4.09 [t, 2H, J = 7.7 Hz, CH_a], 3.98-

3.29 [m, 27H, CH_b, CH_B, CH_A and and Crown ($O-CH_2-$)]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 173.8, 166.8, 166.7, 164.2, 159.8, 159.6, 159.2, 139.0, 138.6, 138.5, 136.8, 136.6, 130.6, 130.5, 130.116.8, 116.7, 77.4, 70.7, 70.3, 70.2, 70.1, 60.8, 49.4; FABMS: *m/z* = 1299 $[M+H]^+$; HRMS : *m/z* = 1299.6003 $[M+H]^+$ (anal. calcd. for $C_{77}H_{83}N_6O_{13}^+$: *m/z* = 1299.6018).

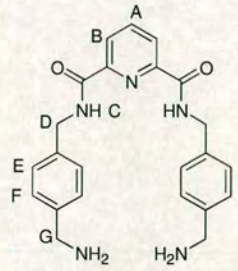
Preparation of {4-[({6-[4-(tert-Butoxycarbonylamino-methyl)-benzylcarbamoyl]-pyridine-2-carbonyl}-amino)-methyl]-benzyl}-carbamic acid tert-butyl ester (43).



2,6-pyridinedicarbonyl dichloride (784 mg, 3.84 mmol) was dissolved in anhydrous $CHCl_3$ (20 mL) and the solution was cooled to 0 °C. Mono-boc xylylene diamine (2.00 g, 8.46 mmol) and Et_3N (861 mg, 8.46 mmol) dissolved in anhydrous $CHCl_3$ (20 mL) were simultaneously added over a period of 2 h using motor-driven syringe pumps at 0 °C. The reaction mixture was stirred for 12 h, concentrated under reduced pressure and then CH_2Cl_2 was added (200 mL). The organic layer was washed twice with 1M NaOH (50 mL) then further washed with brine (sat., 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (gradient elution: CH_2Cl_2 / MeOH 97:3 then CH_2Cl_2 / MeOH 95:5) to furnish compound **43** as a white solid (1.51 g, 65%); mp = 119 °C; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.70 [s, 2H, NH_C], 8.30 [d, 2H, J = 7.8 Hz, Ar-H_B], 7.98 [t, 1H, J = 7.8 Hz, Ar-H_A], 7.19-6.90 [m, 8H, Ar-H_E and Ar-H_F], 5.18 [bt, 1H, NH_H], 4.39 [d, 4H, J = 4.0 Hz, CH_D], 4.14 [d, 4H, J = 5.1 Hz, CH_G], 1.42 [s, 18H, CH_I]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 167.8, 157.8,

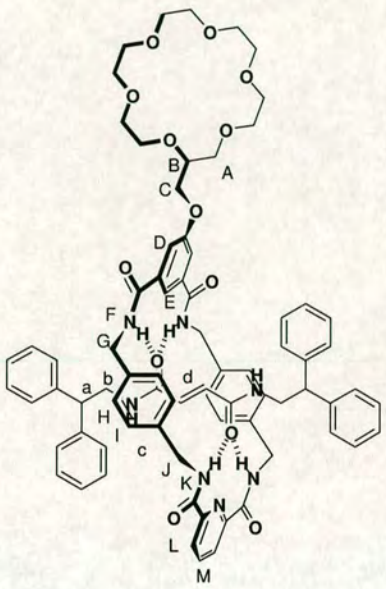
156.2, 148.8, 138.8, 138.2, 127.8, 127.3, 125.1, 79.6, 44.1, 43.1, 28.4; FABMS: *m/z* = 602 $[M+H]^+$; HRMS : *m/z* = 602.4739 $[M+H]^+$ (anal. calcd. for $C_{33}H_{40}N_5O_6^+$: *m/z* = 602.2978).

Preparation of Pyridine-2,6-dicarboxylic acid bis-(4-aminomethyl-benzylamide) (44).



A solution of **43** (1.20 g, 2.0 mmol) in CH_2Cl_2 / TFA 9:1 (40 mL) was stirred for 3 h. The reaction mixture was concentrated under reduced pressure to remove TFA and then CH_2Cl_2 (200 mL) and 1M NaOH (50 mL) were added and the mixture was stirred vigorously for 30 min. The organic layer was separated from the aqueous solution and then dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was triturated twice with diethyl ether to give the product as colourless solid (765 mg, 95%); mp = 180-183 °C; ^1H NMR (400 MHz, d_6-DMSO): δ = 9.70 [t, 2H, J = 6.3 Hz, NH_C], 8.27 [d, 2H, J = 7.7 Hz, Ar-H_B], 8.04 [t, 1H, J = 7.8 Hz, Ar-H_A], 7.29-7.06 [m, 8H, Ar-H_E and Ar-H_F], 4.62 [d, 4H, J = .4 Hz, CH_D], 3.73 [s, 4H, CH_G], 2.40 [bs, 4H, NH_2] ^{13}C NMR (400 MHz, d_6-DMSO/$CDCl_3$ 1:1): δ = 163.0, 156.8, 148.2, 141.5, 138.1, 136.7, 126.5, 123.8, 45.0, 41.6; FABMS: *m/z* = 404 $[M+H]^+$; HRMS : *m/z* = 404.2084 $[M+H]^+$ (anal. calcd. for $C_{23}H_{26}N_5O_2^+$: *m/z* = 404.2086).

Preparation of [2](1,9,16,19,22-Pentaaza-2,8,17,21-tetraaoxo-5-(1,4,7,10,13,16-hexaoxa-Cyclooctadec-2-ylmethoxy)- -3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-(*N,N'–bis*(2,2–diphenyl-ethyl)-(*E*)-butendiamide)-rotaxane (45).



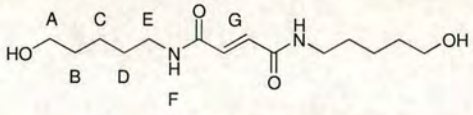
Synthesis of 5-(1,4,7,10,13,16hexaoxa-Cyclooctadec-2-ylmethoxy)-isophthaloyl dichloride (39A).

A stirred solution of **39** (150 mg, 0.31 mmol), $SOCl_2$ (10 mL) and $(COCl)_2$ (0.5 mL) was heated under reflux for 12 hours. The solvent was removed under reduced pressure and the sample was kept overnight under vacuum to remove $SOCl_2$. Then, CH_2Cl_2 was added and the solvent was removed in vacuum. This step has been repeated for 4 times. The crude product was used directly for further reaction.

Thread **4** (100 mg, 0.207 mmol) and compound **44** (100 mg, 0.25 mmol) and Et_3N (86 μL, 0.61 mmol) in anhydrous $CHCl_3$ (150 mL) were stirred vigorously whilst solution of dichloride (155 mg, 0.31 mmol) in anhydrous $CHCl_3$ (20 mL) was simultaneously added over a period of 2 h using motor-driven syringe pumps. After 24 h the resulting

suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography [gradient elution: CH_2Cl_2 / MeOH 95:5 then CH_2Cl_2 / MeOH 90:10] to furnish compound **45** as a white solid (105 mg, 30%); mp = 250 °C (decomp); ^{1}H NMR (400 MHz, $CDCl_3$/MeOH 9:1): δ = 9.88 [t, 2H, J = 6.2 Hz, NH_K], 8.39 [d, 2H, J = 7.8 Hz, Ar-H_L], 8.26 [s, 1H, Ar-H_E], 8.17 [t, 1H, J = 7.8 Hz, Ar-H_M], 8.04 [s, 2H, NH_c], 7.73 [m, 4H, Ar-H_D and NH_F], 7.31-7.02 [m, 20H, Ar-H_{thread}], 6.59-6.47 [m, 8H, Ar-H_H and Ar-H_I], 5.34 [m, 2H, CH_d], 4.30-4.11 [m, 10H, CH_G, CH_J and CH_C], 4.01 [t, 2H, J = 4.8 Hz, CH_a], 3.94-3.62 [m, 24H, CH_b, CH_B, CH_A and Crown (O-CH_2-)]; ^{13}C NMR (400 MHz, $CDCl_3$/MeOH 9:1): δ = 165.9, 165.1, 163.5, 159.2, 158.8, 141.2, 137.1, 134.5, 134.2, 130.4, 128.4, 127.9, 127.6, 127.0, 126.2, 124.2, 116.7, 69.9, 69.5, 49.4, 43.8, 43.7, 28.8; FABMS: *m/z* = 1300 $[M+H]^+$; HRMS : *m/z* = 1300.5989 $[M+H]^+$ (anal. calcd. for $C_{76}H_{82}N_7O_{13}^+$: *m/z* = 1300.5970).

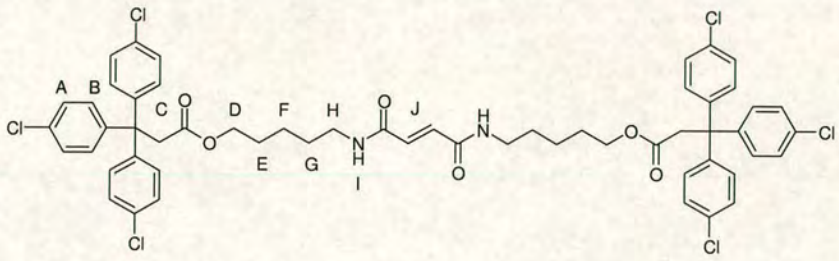
Synthesis of But-2-enedioic acid bis-[(5-hydroxy-pentyl)-amide] (46).



5-Amino-pentan-1-ol (3.0 g, 29.10 mmol) was dissolved in DMF (40 mL) and the solution was cooled to 0 °C. A solution of fumaroyl dichloride in DMF (15 mL) (0.90 g, 5.81 mmol) was added at °C over a period of 2 h using motor-driven syringe pumps. The reaction mixture was stirred for 2 h, concentrated under reduced pressure and then CH_2Cl_2 was added (300 mL). To the organic layer a solution of 3M HCl in diethyl ether was added to precipitate the excess of unreacted 5-Amino-pentan-1-ol and the solid was filtrated through a plug of celite. The plug was washed twice with warm DMF. The solvent was removed under reduced pressure and the crude product was isolated without further purification. (1.16 g, 70%); mp = 220 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.36 [t, 2H, J = 5.6 Hz, NH_F], 6.80 [s, 2H, CH_G], 3.36 [t, 4H, J = 6.4 Hz, CH_A], 3.12 [q, 4H, J = 6.0 Hz, CH_E], 1.47-1.34 [m, 8H, CH and CH_B and CH_D], 1.32-1.21 [m, 4H,

CH_C]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 163.6, 132,6, 60.6, 32.2, 28.8, 28.6, 23.0; FABMS: *m/z* = 286 $[M]^+$; HRMS : *m/z* = 286.1023 $[M]^+$ (anal. calcd. for $C_{14}H_{26}N_2O_4^+$: *m/z* = 286.1892).

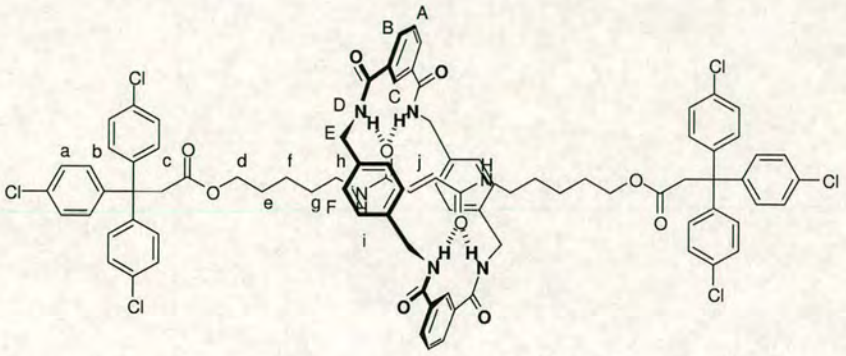
Synthesis of 3,3,3-Tris-(4-chloro-phenyl)-propionic acid 5-(3-{5-[3,3,3-tris-(4-chloro-phenyl)-propionyloxy]-pentylcarbamoyl}-acryloylamino)-pentyl ester (47).



3,3,3-Tris-(4-chloro-phenyl)-propionic acid (1.53 g, 3.77 mmol) was dissolved in CH_2Cl_2 (30 mL) and the solution was cooled to 0 °C. EDCI·HCl (984 mg, 5.13 mmol), 4-DMAP (625 mg, 5.12 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 30 min. A solution of **46** (20 mL) (490 mg, 1.71 mmol) was added to the activated acid. The reaction mixture was stirred for 4 h, concentrated under reduced pressure and then CH_2Cl_2 was added (300 mL). The organic layer was washed only once with 1M HCl (50 mL), with $NaHCO_3$ (sat. aq., 50 mL) then the organic layer was concentrated under reduced pressure. The crude material was purified by flash chromatography [eluant: CH_2Cl_2 / MeOH 98:2] to furnish compound **47** as a colorless solid (1.50 g, 84%); mp = 134 °C; ^1H NMR (400 MHz, $CDCl_3$/MeOH 9:1): δ = 8.01 [t, 2H, J = 5.6 Hz, NH_I], 7.23-7.16 [m, 12H, Ar-H_A], 7.12-7.04 [m, 12H, Ar-H_B], 6.71 [s, 2H, CH_J], 3.75 [t, 4H, J = 6.5 Hz, CH_D], 3.59 [s, 4H, CH_C], 3.19 [m, 4H, CH_H], 1.51-1.26 [m, 8H, CH_E and CH_G], 1.19-1.05 [m, 4H, CH_F]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 170.4, 164.2, 144.2, 133.0, 132.6, 130.3, 128.2, 64.3, 54.6, 46.0, 39.6, 29.0, 28.0, 23.2;

FABMS: *m/z* = 1059 $[M+H]^+$; HRMS : *m/z* = 1061.2086 $[M+H]^+$ (anal. calcd. for $C_{56}H_{55}N_2O_6Cl_6^+$: *m/z* = 1061.2191).

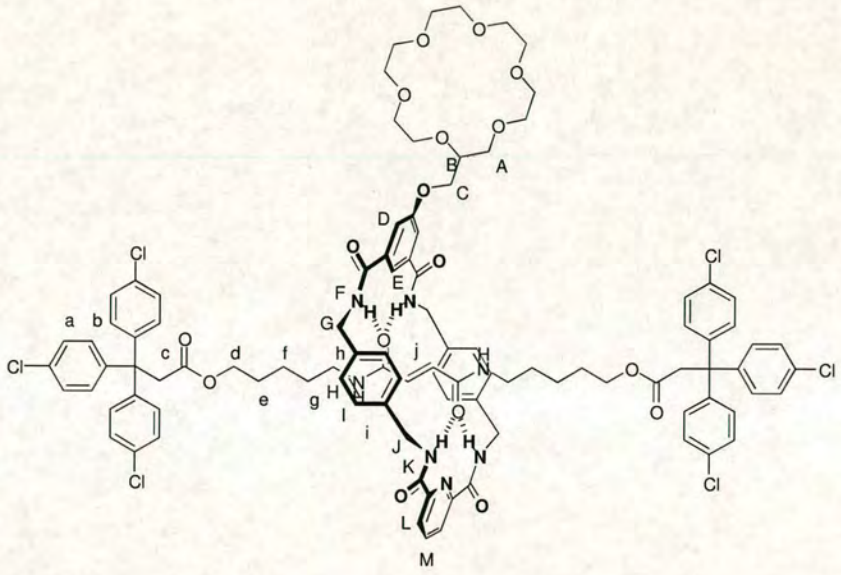
Preparation of [2]-(1,4,7,14,17,20-Hexaaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacoxane)-((E)3,3,3-Tris-(4-chloro-phenyl)-propionic acid 5-(3-{5-[3,3,3-tris-(4-chloro-phenyl)-propionyloxy]-pentylcarbamoyl}-acryloylamino)-pentyl ester)-rotaxane (48).



Thread **47** (100 mg, 0.1 mmol) in anhydrous $CHCl_3$ (50 mL) was stirred vigorously whilst solutions of para-xylylene diamine (197 mg, 1.45 mmol) and Et_3N (295 mg, 2.90 mmol) in anhydrous $CHCl_3$ (20 mL) and isophthaloyl dichloride (294 mg, 1.45 mmol) in anhydrous $CHCl_3$ were simultaneously added over a period of 2h using motor-driven syringe pumps. After a further 4h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography [elution: CH_2Cl_2 / methanol 95:5] to furnish compound **48** as a white solid (151 mg, 95%); mp = 250 °C (decomp); ^{1}H NMR (400 MHz, $CDCl_3$/MeOH 9:1): δ = 8.67 [br t, 2H, Ar-H_C], 8.11 [dd, 4H, J = 1.60Hz, J = 7.7 Hz, Ar-H_B], 7.57 [t, 2H, J = 7.8 Hz, Ar-H_A], 7.20-7.15 [m, 12H, Ar-Ha], 7.08-7.02 [m, 12H, Ar-H_b], 6.97 [s, 8H, Ar-H_F], 5.64 [s, 2H, CH_j], 4.38 [s, 8H, CH_E], 3.73 [t, 4H, J = 6.5 Hz, CH_d], 3.56 [s, 4H, CH_c], 3.00 [t, 4H, J = 7.4 Hz, CH_h], 1.39-0.72 [m, 12H, CH_e and CH_g and CH_f]; ^{13}C NMR (400 MHz, $CDCl_3$/MeOH 9:1): δ = 170.4, 167.0, 165.3, 143.8, 136.7, 133.4, 133.0, 132.3, 131.1, 130.0, 128.6, 128.2, 127.9, 124.7, 64.0, 54.4, 45.7, 43.6, 39.3, 28.4,

27.6, 23.0; FABMS: *m/z* = 1596 $[M+H]^+$; HRMS : *m/z* = 1599.4731 $[M+H]^+$ (anal. calcd. for $C_{88}H_{89}Cl_6N_6O_{10}^+$: *m/z* = 1599.4771).

Preparation of [2](1,9,16,19,22-Pentaaza-2,8,17,21-tetraaoxo-5-(1,4,7,10,13,16-hexaoxa-Cyclooctadec-2-ylmethoxy)-3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-((E)3,3,3-Tris-(4-chloro-phenyl)-propionic acid 5-(3-{5-[3,3,3-tris-(4-chloro-phenyl)-propionyloxy]-pentylcarbamoyl}-acryloylamino)-pentyl ester)-rotaxane (49).

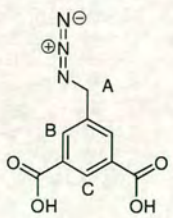


A stirred solution of **39** (370 mg, 0.77 mmol), $SOCl_2$ (10 mL) and $(COCl)_2$ (0.5 mL) was heated under reflux for 12 hours. The solvent was removed under reduced pressure and the sample was kept overnight under vacuum to remove $SOCl_2$. Then, CH_2Cl_2 was added and the solvent was removed in vacuum. This step has been repeated for 4 times. The crude product was used directly for further reaction.

Thread **47** (105 mg, 0.101 mmol) and compound **44** (228 mg, 0.81 mmol) and Et_3N (226 μL, 1.60 mmol) in anhydrous $CHCl_3$ (30 mL) were stirred vigorously whilst solution of **39A** (370 mg, 0.81 mmol) in anhydrous $CHCl_3$ (20 mL) was simultaneously added over

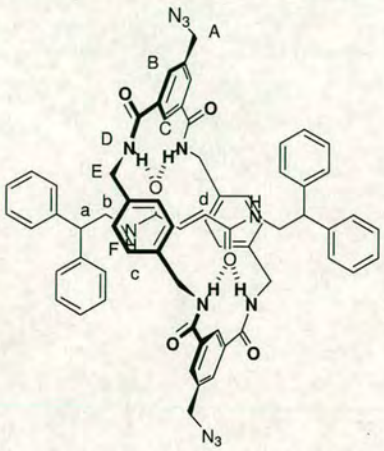
a period of 2 h using motor-driven syringe pumps. After 24 h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography [gradient elution: CH_2Cl_2 / MeOH 95:5 then CH_2Cl_2 / MeOH 90:10] to furnish compound **49** as a colourless solid (140 mg, 70%); mp = 220-224 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.71 [br t, 2H, NH_K], 8.44 [s, 1H, Ar-H_E], 8.42 [s, 2H, Ar-H_L], 8.06 [t, 1H, J = 7.8 Hz, Ar-H_M], 8.02 [br t, 2H, NH_F], 7.70 [s, 2H, Ar-H_D], 7.25-7.17 [m, 12H, Ar-H_a], 7.14-7.05 [m, 12H, Ar-H_b], 7.01 [m, 8H, Ar-H_H and Ar-H_I], 6.32 [br s, 2H, NH_c], 5.72 [s, 2H, CH_j], 4.52 [br d, 4H, CH_G or CH_J], 4.41 [br d, 4H, CH_G or CH_J], 4.30 [br d, 2H, CH_C], 3.95 [br t, 1H, CH_B], 3.88-3.40 [m, 28H, CH_A, and Crown (O-CH_2-) and CH_c], 3.01 [m, 4H, CH_d], 2.13 [m, 4H, CH_h], 1.48-1.16 [m, 8H, CH_e and CH_g], 1.15- 0.93 [m, 4H, CH_f]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 170.3, 166.50, 165.1, 163.9, 159.1, 149.4, 144.2, 138.3, 137.1, 135.6, 132.6, 130.4, 128.8, 12.7, 128.2, 125.2, 117.3, 70.4, 70.0, 69.8, 64.3, 54.6, 46.0, 44.3, 43.0, 39.9, 28.8, 28.0, 23.4; FABMS: *m/z* = $[M+Na]^+$ 1906; HRMS : *m/z* = 1906.5546 $[M+Na]^+$ (anal. calcd. for $C_{100}H_{103}Cl_6N_7O_{17}Na^+$: *m/z* = 1906.5439).

Preparation of 5-Azidomethyl-isophthalic acid (50).



5-Azidomethyl-isophthalic acid was prepared as reported in literature by Toone and coworkers,[3] starting from benzenetricarboxylic acid.

Preparation of [2](1,9,16,24-Tetraaza-2,8,17,23-tetraoxo-5,20-azidomethyl-3,7,11,14,18,22,26,29-tetrabenzocyclohexacosane))-(*N,N'–bis*(2,2–diphenyl-ethyl)-(*E*)-butendiamide)-rotaxane (51).

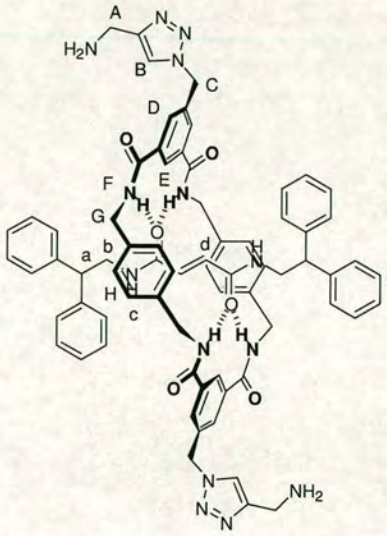


A stirred solution of 5-Azidomethyl-isophthalic acid **50** (500 mg, 2.26 mmol), $SOCl_2$ (10mL) and $(COCl)_2$ (0.5 mL) was heated under reflux for 12 hours. The solvent was removed under reduced pressure and the sample was kept overnight under vacuum to remove $SOCl_2$. Then, CH_2Cl_2 was added and the solvent was removed in vacuum. This step has been repeated for 4 times. The crude product was used directly for further reaction.

Thread **4** (155 mg, 0.33 mmol) in anhydrous $CHCl_3$ (50 mL) and CH_3CN (10 mL) was stirred vigorously whilst solutions of para-xylylene diamine (268 mg, 1.97) and TEA (0.61 mL, 4.36 mmol) in anhydrous $CHCl_3$ (20 mL) and 5-Azidomethyl-isophthaloyl dichloride (480 mg, 2.17 mmol) in anhydrous $CHCl_3$ were simultaneously added over a period of 2h using motor-driven syringe pumps. After a further 4h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography [elution: CH_2Cl_2 / MeOH 97:3] to furnish compound **51** as a white solid (258 mg, 70%); mp = 250 °C (decomp); ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.60 [s, 2H, Ar-H_C], 8.51 [t, 2H, J = 5.5 Hz, NH_c], 8.17 [t, 4H, J = 4.8 Hz, NH_D], 8.09 [s, 4H, Ar-H_B], 7.29-7.12 [m, 20H, Ar-H_{thread}], 6.66 [s, 8H,

Ar-H_F], 5.64 [s, 2H, CH_d], 4.71 [s, 4H, CH_A], 4.21 [d, 8H, J = 4.5 Hz, CH_E], 4.09 [t, 2H, J = 7.7 Hz, CH_a], 3.66 [d, 4H, J = 7.3 Hz, CH_b]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 165.4, 165.3, 142.6, 141.3, 137.1, 136.3, 135.2, 134.7, 130.6, 128.5, 127.7, 126.6, 125.0, 53.0, 49.9, 43.3, 43.2; FABMS: *m/z* = 1117 $[M+H]^+$; HRMS : *m/z* = 1117.4831 $[M+H]^+$ (anal. calcd. for $C_{66}H_{61}N_{12}O_6^+$: *m/z* = 1117.4837).

Preparation of [2](1,9,16,24-Tetraaza-2,8,17,23-tetraoxo-5,20-(4-aminomethyl-[1,2,3]triazol-1-ylmethyl)-3,7,11,14,18,22,26,29-tetrabenzocyclohexacosane))-(*N,N'–bis*(2,2–diphenyl-ethyl)-(*E*)-butendiamide)-rotaxane (52).



Rotaxane **51** (73 mg, 0.065 mmol), Tetrakis-acetonitrile-Copper(I)-hexafluorophosphate (4.8 mg, 0.013 mmol) in $CHCl_3$ (15 mL) and MeOH (5 mL) were stirred vigorously whilst mono-propargyl amine (35.8 mg, 0.65 mmol) was added. The reaction mixture was stirred for 18 h, concentrated under reduced pressure. Aqueous washes were avoided because of the poor solubility of the rotaxane in organic solvents. The crude material was purified by flash chromatography [eluant: CH_2Cl_2 / MeOH 90:10 and 1% of NH_3 (aq)] to furnish compound **52** as a colorless solid (76 mg, 95%); mp = 250 °C

(decomp); ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.58 [s, 2H, Ar-H_E], 8.50 [t, 2H, J = 5.0 Hz, NH_{thread}], 8.13 [t, 4H, J = 4.7 Hz, NH_F], 8.06 [s, 6H, Ar-H_D and CH_B], 7.27-7.11 [m, 20H, Ar-H_c], 6.63 [s, 8H, Ar-H_H], 5.79 [s, 4H, CH_C], 5.60 [s, 2H, CH_D], 4.19 [d, 8H, J = 4.6 Hz, CH_G], 4.07 [t, 2H, J = 7.6 Hz, CH_a], 3.80 [s, 4H, CH_A], 3.64 [t, 4H, J = 6.3 Hz, CH_b]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 165.3, 165.2, 142.6, 137.4, 136.2, 134.7, 132.4, 129.2, 128.5, 127.7, 126.5, 124.9, 122.5, 52.2, 49.8, 43.6, 43.3, 36.5; FABMS: *m/z* = 1227 $[M+H]^+$; HRMS : *m/z* = 1227.5688 $[M+H]^+$ (anal. calcd. for $C_{72}H_{71}N_{14}O_6^+$: *m/z* = 1227.5681).

X-ray crystallographic data for compound 36 (DMF/Et_2O).

Empirical formula: C74 H77 N9 O10; Formula weight: 1252.45; Wavelength: 0.71073 A; Temperature: 150(2) K; Crystal system: Monoclinic; Space group: P21/c; Unit cell dimensions: a = 15.0057(4) Å, α = 90.00°; b = 23.5226(7) Å, β = 101.788(2)°; c = 18.8739(5) Å, γ = 90.00°; Volume: 6521.5(3) $Å^3$; Number of reflections for cell: 6718 ($2 < \theta < 29.5°$); Z: 4; Density (calculated): 1.276 Mg/m^3; Absorption coefficient: 0.086 mm^{-1}; F(000): 2656; Crystal size: 0.95 x 0.09 x 0.09 mm^3; Instrument: Bruker Smart Apex CCD; Theta range for data collection: 1.40 to 22.57°; Index ranges: -16<=h<=15, 0<=k<=25, 0<=l<=20; Reflections collected: 71805; Independent reflections: 8601 [R(int) = 0.1105]; Absorption correction: Multiscan (Tmin= 0.787, Tmax=0.992); Solution: direct (SHELXS-97 (Sheldrick, 1990)); Refinement type: Full-matrix least-squares on F^2; Program used for refinement: SHELXL-97; Hydrogen atom placement: geom.; Hydrogen atom treatment: riding/rotating group (Me in DMF); Data / restraints / parameters: 8601/820/840; Goodness-of-fit on F^2: 1.043; Conventional R [F>4sigma(F)] R1 = 0.0501 [5410 data]; Weighted R (F^2 and all data): wR2 = 0.1305; Final maximum δ/σ: 0.001; Largest diff. peak and hole: 0.275 and -0.271 $e.A^3$.

X-ray crystallographic data for compound 51 (DMF/Et_2O).

Empirical formula: C66 H64 N12 O8; Formula weight: 1153.29; Temperature: 93(2) K; Wavelength: 0.71073 Å; Crystal system: Triclinic; Space group: P-1; Unit cell dimensions: a = 10.3105(12) Å, α = 94.283(7)°; b = 10.7700(12) Å, β = 107.026(7)°; c = 15.3975(19) Å, γ = 112.783(6)°; Volume: 1472.6(3) $Å^3$; Z:1; Density (calculated): 1.300 Mg/m^3; Absorption coefficient: 0.088 mm-1; F(000): 608; Crystal size:0.150 x 0.100 x 0.010 mm^3; Theta range for data collection: 2.10 to 25.35°; Index ranges: -9<=h<=12, -12<=k<=12, -17<=l<=16 Reflections collected: 10465; Independent reflections: 4686 [R(int) = 0.0538]; Completeness to theta = 25.00°: 88.3 % Absorption correction: Multiscan; Max. and min. transmission:1.0000 and 0.3112; Refinement method: Full-matrix least-squares on F^2; Data / restraints / parameters: 4686 / 5 / 409; Goodness-of-fit on F^2:1.083; Final R indices [I>2sigma(I)]: R1 = 0.0537, wR2 = 0.1277 R indices (all data): R1 = 0.0643, wR2 = 0.1362; Largest diff. peak and hole: 0.239 and -0.216 e.$Å^{-3}$.

X-ray crystallographic data for compound 51 ($MeOH/CH_2Cl_2$).

Empirical formula: C66 H60 N12 O6; Formula weight: 1117.26; Temperature: 93(2) K; Wavelength: 0.71073 Å; Crystal system: Triclinic; Space group P-1; Unit cell dimensions: a = 9.4352(11) Å, α = 84.310(6)°; b = 11.1836(12) Å, β = 74.336(6)°; c = 14.2622(16) Å, γ = 77.168(6)°; Volume: 1411.6(3) $Å^3$; Z:1; Density (calculated): 1.314 Mg/m^3; Absorption coefficient: 0.087 mm^{-1}; F(000): 588; Crystal size: 0.180 x 0.050 x 0.030 mm^3; Theta range for data collection: 2.34 to 25.35°; Index ranges: -8<=h<=11, -7<=k<=13, -16<=l<=17; Reflections collected: 9280; Independent reflections: 4332 [R(int) = 0.0899]; Completeness to theta = 25.00°: 84.9 %; Absorption correction: Multiscan; Max. and min. transmission: 1.0000 and 0.0607; Refinement method: Full-matrix least-squares on F^2; Data / restraints / parameters: 4332 / 3 / 392; Goodness-of-fit on F^2: 1.061; Final R indices [I>2sigma(I)]: R1 = 0.1074, wR2 = 0.2609; R indices (all data): R1 = 0.1198, wR2 = 0.2751; Largest diff. peak and hole: 0.951 and -0.362 e.$Å^{-3}$.

X-ray crystallographic data for compound 52 (DMF/Et_2O).

Empirical formula: C84 H98 N18 O10; Formula weight: 1519.80; Temperature: 173(2)K; Wavelength: 1.54178 Å; Crystal system: Triclinic; Space group: P-1; Unit cell dimensions: a = 9.724(8) Å, α = 65.00(3)°; b = 14.527(3) Å, β = 87.81(3)°; c = 16.046(10) Å, γ = 79.66(3)°; Volume: 2019(2) $Å^3$; Z: 1; Density (calculated): 1.250 Mg/m^3: Absorption coefficient: 0.682 mm^{-1}; F(000): 808; Crystal size 0.1000 x 0.0300 x 0.0300 mm^3; Theta range for data collection: 3.04 to 67.96°; Index ranges: -11<=h<=10, -17<=k<=17, -19<=l<=19; Reflections collected: 26461; Independent reflections: 6748 [R(int) = 0.1008]; Completeness to theta = 25.00°: 92.0 %; Absorption correction: Multiscan; Max. and min. transmission: 1.0000 and 0.3967; Refinement method: Full-matrix least-squares on F^2; Data / restraints / parameters: 6748 / 3 / 523; Goodness-of-fit on F^2: 1.057; Final R indices [I>2sigma(I)]: R1 = 0.0761, wR2 = 0.2009; R indices (all data): R1 = 0.0951, wR2 = 0.2261; Extinction coefficient: 0.0043(7); Largest diff. peak and hole: 0.717 and -0.401 $e.Å^{-3}$.

References.

[1] F. G. Gatti, D. A. Leigh, S. A. Nepogodiev, A. M. Z. Slawin, S. J. Teat, J. K. Y. Wong, *J. Am. Chem. Soc.* **2001**, *123*, 5983-5989.

[2] T. J. Kidd, D. A. Leigh, A. J. Wilson, *J. Am. Chem. Soc.* **1999**, *121*, 1599-1600.

[3] S. M. Dimick, S. C. Powell, S. A. McMahon, D. N. Moothoo, J. H. Naismith, E. J. Toone, *J. Am. Chem. Soc* **1999**, *121*, 10286-10296.

3 Synthesis of Membrane Spanning Rotaxanes

3.1 Introduction to ion transport across biological membranes.

The flow of ions and small neutral molecules either into or out of a cell is essential for the prosperity of any biological system. The molecular and ionic composition of the intracellular medium is regulated by a selective transport system while membranes control and prevent the random flow of chemicals between cells and their own environment. Biological membranes are complex and, as such, their study is difficult. This may be due to the presence of a number of elaborate structural features, such as: a) complex transmembrane proteins, b) phospholipids of various chemical structures and compositions, as well as steroids and numerous other structural and regulatory compounds that make up the cell membrane (Figure 1).

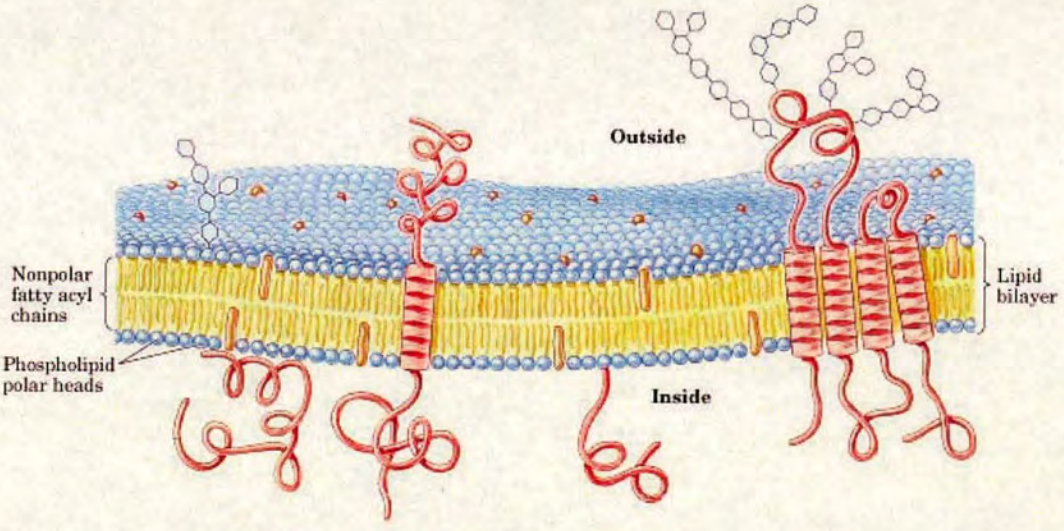


Figure 1. Structure of a biological cell membrane
(picture taken from http://www.uoguelph.ca/~fsharom/research/research.html).

Proteins in particular are the most important constituent of a membrane and their purpose is to act as gated channels, receptors or catalytic enzymes. Proteins can (a) alter the flexibility of membranes, (b) conduct chemical reactions, (c) communicate either intra- or inter-cellularly, and perhaps most importantly (d) regulate the passage and concentrations of ions.

The study of biological membranes *in vitro* may be simplified by using phospholipids monomers, usually called liposomes.[1-4] Membrane monomers form bilayers where their polar head groups are oriented toward the aqueous phase and their hydrophilic hydrocarbon chains form the interior of the membrane (Figure 2a).

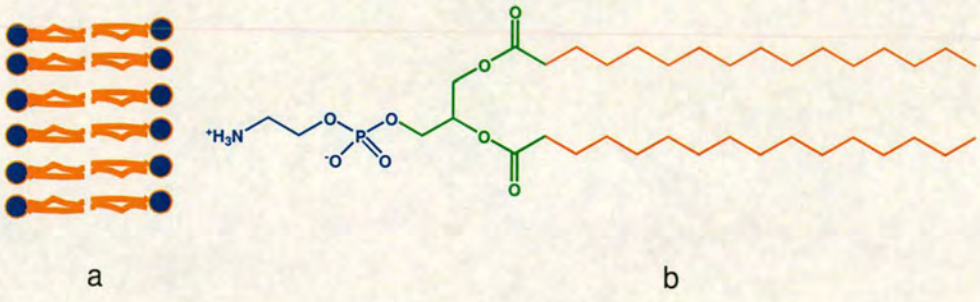


Figure 2. (a) Structure of the lipid bilayers and (b) a membrane monomer.

Typical membrane-forming liposomes are characterized by three different regions (Figure 2b): (a) a non-polar tail, normally constituted by two alkyl chains with physical properties close to hexane, having a dielectric constant (ε) of about 2, (b) a polar head group with anionic phosphate groups with a hydrophilic nature expected to interact with the aqueous medium that surrounds the cell having a value of ε of about 80, quite close to the value of water, and (c) a midpolar glycerol region connecting the two, with an ε of about 30. On forming a membrane it becomes clear that, due to the low dielectric constant of the non polar tail that forms what is called the "apolar hydrophobic interior", a considerable amount of energy is required to transfer an ion such sodium or potassium from one side of the membrane to the other, across the hydrophobic interior.[5] The energy required due to the impermeability of bilayers is much higher than the mean thermal energy.

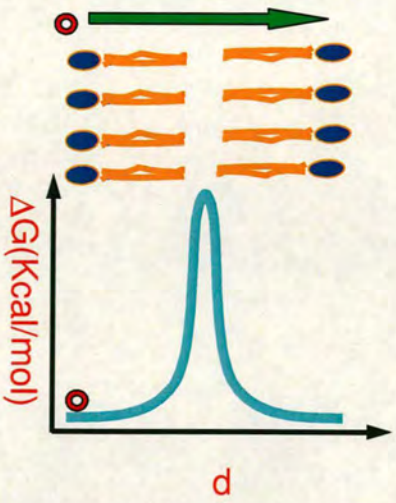


Figure 3. High energy barrier for the passage of ions.

Figure 3 shows an energy diagram illustrating the lipid portion of the membrane together with it's extremely high energy barrier for the

passage of ions. Theoretically, the energy required for an ion to pass from an aqueous phase through a hydrophobic medium is calculated by measuring the electrostatic interaction between the ion and the medium, and is about 180 kJ/mol.[6] This excludes the possibility of a simple translocation mechanism for ions to cross a membrane. However, despite this barrier, ions do migrate across membranes even in the absence of special molecules able to assist the transport. Ion permeability in pure phospholipids vesicles is difficult to measure because the rates of transport are very small and other effects, such as vesicle fusion followed by dumping of contents, may obscure results from single ion passage. The mechanisms of unassisted transport of ions across membranes are discussed in more detail below.

3.2 Mechanisms for ionic permeation.

Two main transport mechanisms for ionic permeation have been proposed.[6-12]

- the partition model
- the pore model

These models are detailed in the following sections.

3.2.1 The partition model.[7, 11, 12]

In this mechanism, the ion samples the interior hydrocarbon phase of the bilayer as shown in Figure 4. The membrane is modeled in theoretical calculations as an energy barrier that the permeating cation (or anion) must overcome, rather than a separate impermeable phase. The permeation is described by a solubility diffusion theory based on the energy required to transfer ions from the aqueous phase to the hydrocarbon membrane core. Electrostatic considerations are primary concerns of this theory. The ion is described as a sphere of radius a with a charge q distributed uniformly over its surface, while the bilayer is considered a homogeneous liquid with a macroscopic dielectric constant ε. The Gibbs free energy transfer $\Delta G(tr)$ required for an ion to be transferred from an aqueous phase to a hydrocarbon phase can be simply described by the sum of the following contributes:

$$\Delta G(tr) = \Delta G(el) + \Delta G(solv) + \Delta G(si)$$

where ΔG(el) is the electrostatic contribution (which is the most important), ΔG(solv) is the hydrophobic effect, and ΔG(si) comprises interaction between the ion and the solvent as well as formation of dipoles and hydrogen-bonds.

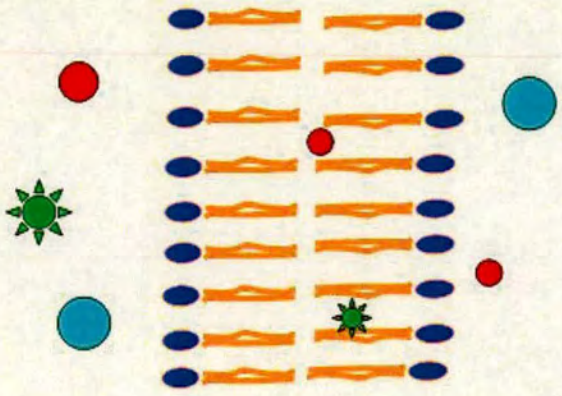


Figure 4. The partition mode.

Ions cross the membrane at a rate proportional to the concentration of ions at the bilayer surface, and the depth of permeation is related to the internal energy of the colliding ion.

3.2.2 The pore model.[6, 8, 9, 11]

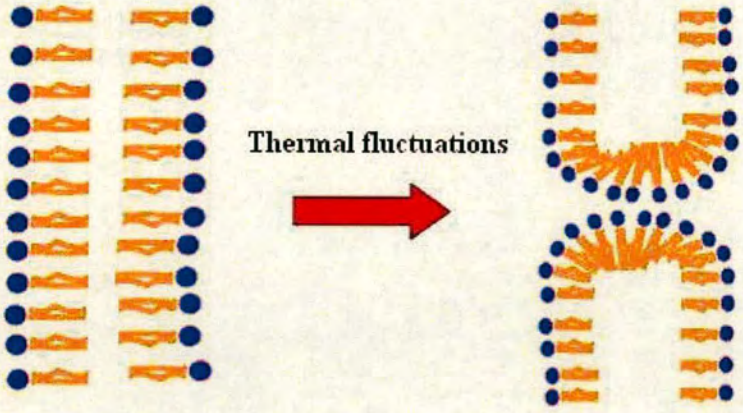


Figure 5. The pore model.[6, 8, 9, 11]

This model is based on the assumption that temporary holes are produced in the bilayer by thermal fluctuations, and the polar head groups rearrange in such a way that they form the walls of the pore in which the surface is lined with head groups (Figure 5). Ion permeation is permitted through what are called "hydrated transient defects" which allow the ions to bypass over the high energy barrier associated with the partition mechanism. The hydrocarbon portion of the lipid monomer remains exposed to the aqueous medium and the permeating ion interacts only with polar head groups at the surface of the bilayer. Theoretical estimates have postulated the formation of an average of 1 pore every 2000 lipid molecules. By comparison, the Partition model is a considerably lower probability process, whereas the Pore model, given the higher rate of ions that is exchanged between the two interfaces, is more plausible.

3.3 Introduction to ion transporters.

In vivo, complex proteins modulate the membrane permeability of ions crossing the lipid bilayer by acting as gated channels. The details behind the chemical mechanisms by which transport and selectivity are achieved are still somewhat a mystery although enormous strides have been made recently towards better understanding the function of transmembrane protein channels. [13]

Two main families of ion transporters may be classed as follows:

- carrier molecules
- channel compounds

3.3.1 Carrier Molecules

A carrier compound[14] is a molecule that associates with the ion at one surface of the membrane and transports it through the membrane to the other by diffusion (as a 'lipophilic' complex), drastically reducing the activation energy needed for translocation. The transport of ions through the membrane by a carrier molecule is not a simple diffusion process. The mechanism may be viewed as avoiding a barrier

of high activation energy. The action of a mobile carrier in the lipid bilayer is presented as follows (Figure 6):

I. binding of the ion by the carrier and stripping of its hydration layer near the surface of the bilayer
II. migration of the lipophilic complex to the opposite interface
III. dissociation of the complex and liberation of the ion on the opposite side of the bilayer
IV. diffusion of the carrier molecule to the original membrane surface

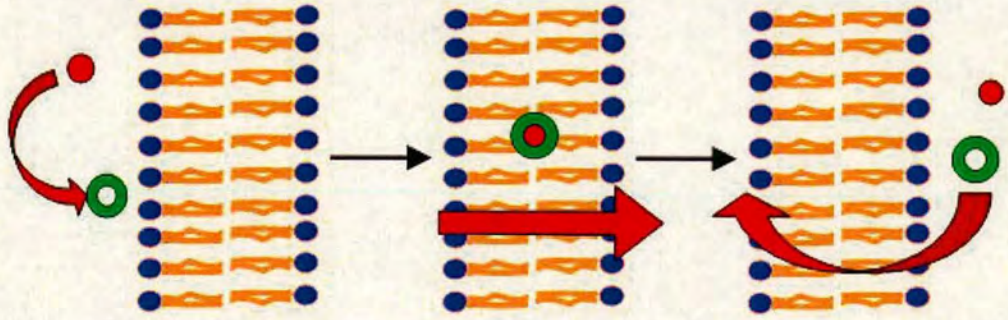


Figure 6. Carrier-Mediated transport of ions.

The most studied and best characterized natural carriers to date are macrocyclic compounds such as monensin and valinomycin. The latter selectively binds K^+ over Na^+ and the complex moves through the membrane to shuttle the ion from one side to the other. Importantly, the rate of transport is proportional to the diffusion rates of the complex through the membrane. Over the past 40 years a remarkable amount of carrier molecules have been synthesized and studied in the hope of mimicking to some extent what nature does. There are a wide variety of synthetic carrier molecules known such as crown ethers, azacrowns or cryptands[15, 16] and complex molecules such as calix[4]arenes and related ionophores.[17, 18] So far, the most sophisticated molecular carrier reported is the "molecular umbrella" **53** proposed by Regen and coworkers, where macromolecules such as oligonucleotides are transported across a phopholipid bilayer while attached to a shielding cholesterol bearing moiety[19] (Figure 7).

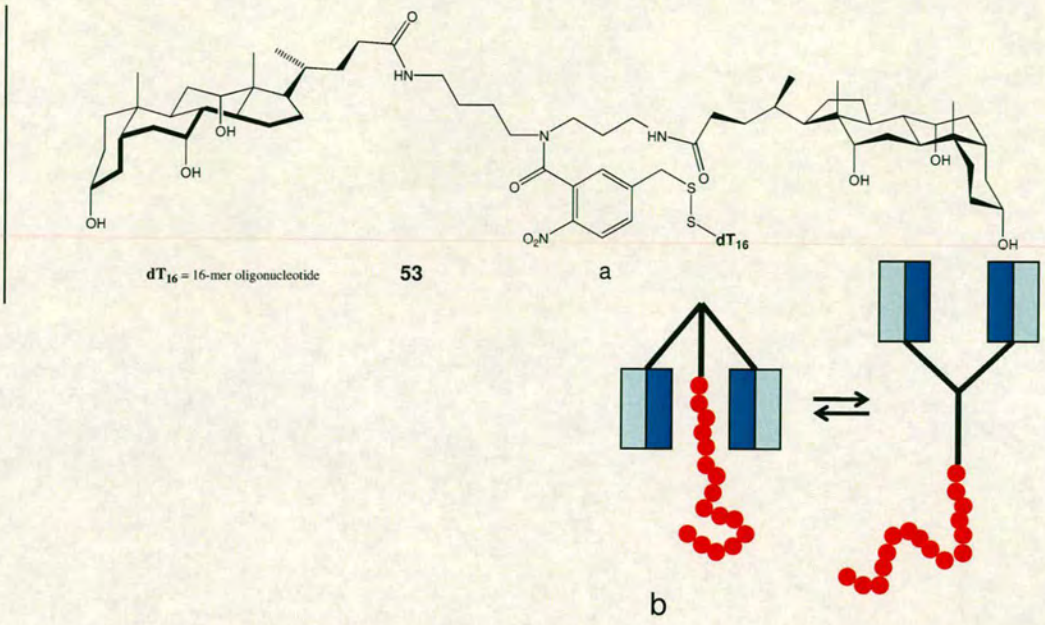


Figure 7. a) Regen's molecular umbrella and b) its mechanism of action.[19]

3.3.2 Channel compounds

Channel molecules are different from carriers in that they "span" the entire membrane, creating a tunnel-like pathway for ions (Figure 8a).

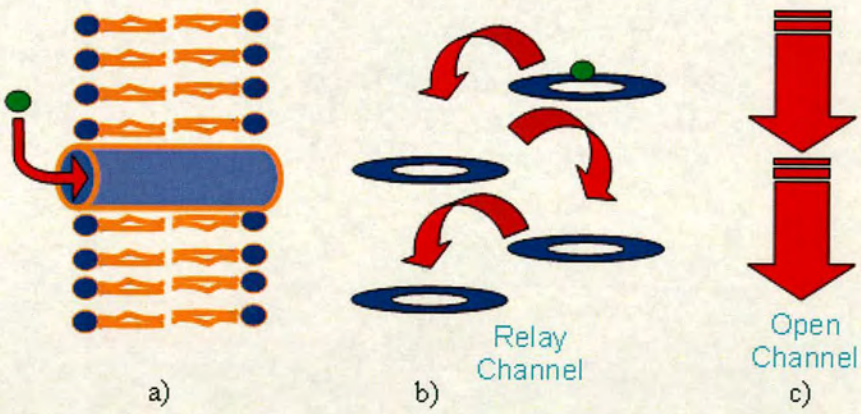
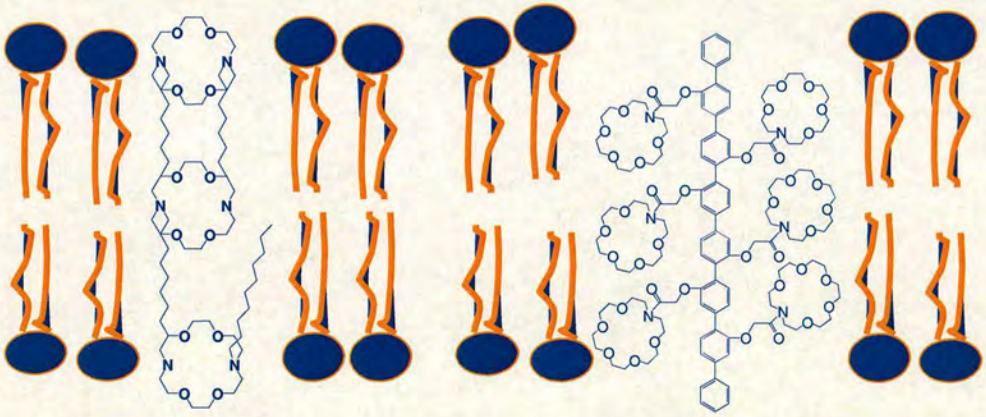


Figure. 8. (a) How a channel compound works, (b) a Relay Channel and (c) an Open Channel.

The channel is resident in the bilayer and forms pores through which ions and water may flow from one side to the other by simply interacting with the hydrophilic

groups attached to the walls of to the channel; ion interaction with the hydrophobic part of the bilayer is minimal (Figure 8a). Natural transporter proteins are highly regulated, in contrast to *most small molecule ion channels which are effectively unregulated and open or close randomly due to movement within the bilayer.* Gramicidin, a natural pentapeptide is one such example.[20]

Understanding how channel compounds work and, more importantly, and perhaps using this knowledge to build new synthetic channels which feature the control of their natural counterparts, is a considerable challenge.[21-23] The synthesis of a controllable transmembrane transporter molecule is the essence of this thesis. There are two main families of channel compounds which differ only by the extent of interaction between solute and channel wall. The first important type of interaction is found in relay channels (Figure 8b) In these systems the solute briefly occupies specific binding sites of the channel as it passes through it. These systems often exhibit high solute selectivity. Two recent examples of relay channels come from the works of Gokel (Figure 9a) and Matile (Figure 9b).[24, 25]



9a 9b

Figure 9. Examples of synthetic relay channels (a) Gokel's system and (b) Matile's system.[24, 25]

The second type relies on a simple mechanism whereby the solute passes through a relatively large open channel (Figure 8c). The only selection criteria in this case is the size of the solute. Channels may promote the passage of ions passively, driven by an electrochemical gradient, or actively against a gradient.[26] An example of an

active channel is the synthetic system proposed by Kobuke and coworkers wherein K^+ moves generally from phosphate to carboxylate groups in the channel, demonstrating a rectified behaviour. [27] Furthermore, some channels may have sophisticated switching mechanisms; that is the property of being gated (on and off states) allowing the flow of ions to be regulated by external stimuli (ie. chemical, electrical, or mechanical triggers).[28] Ion transport in channels is 10^3-10^4 times more efficient than their carrier counterparts. Such structures are capable of transporting 10^8 ions per second across phospholipid bilayers.[29]

3.4 Design of a Membrane Spanning Rotaxane.

As mentioned earlier, ion transporters are complicated systems and understanding their mechanisms of action at the molecular level is a significant challenge. How the substrate actually moves through the bilayer in these systems is less understood than what regulates the transport. Synthetic transport systems may help in understanding some aspects of the biological systems, in addition to offering possible immediate utility as pharmaceutical agents. An example of host-[2]rotaxane which can be used as a cellular transport agent has been synthesized by Smithrud and co-workers.[30, 31] Their system functions as a carrier molecule in that the [2]rotaxane binds a variety of amino acids and dipeptides and the bound complex shuttles across the bilayer.

In this chapter we describe the design and synthesis of novel membrane spanning [2]rotaxanes that may find utility as molecular carriers. The design is based on a photo or thermally driven molecular shuttle previously described by Leigh and co-workers[32] in which biased movement of a macrocycle is achieved over a relatively large distance between two stations (1.5 nm). The trigger responsible for initiating the movement in the shuttle is photochemical and thermal interconversion of fumaramide and maleamide groups, respectively (Figure 10).

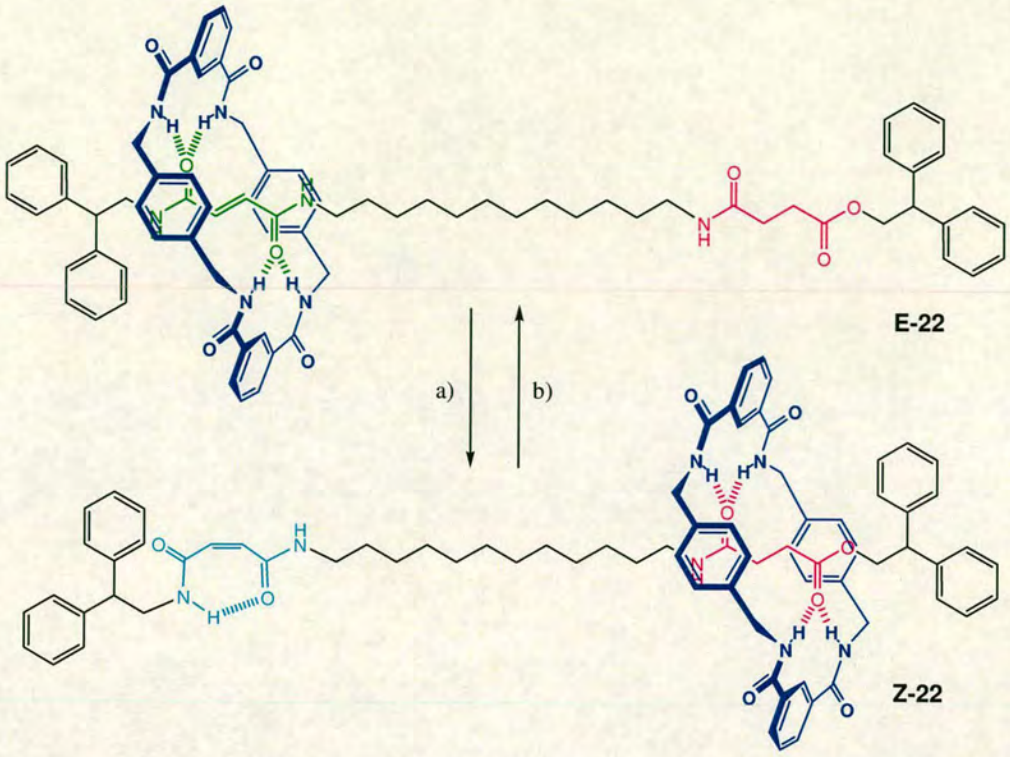


Figure 10. Leigh's light and heat switchable molecular shuttle **22**. a) λ = 254 or 312 nm; b) heat or piperidine.[32]

The membrane spanning rotaxane is a hybrid molecule containing both a carrier compound and a channel like molecule; a system where the carrier is essentially attached on a rail. The two parts of the system are: a) the thread which acts like a pseudo-channel and spans the bilayer, and b) the macrocycle which plays the role of the carrier, transporting the solute across the bilayer. The shuttling of the macrocycle (which contains the carrier in the present system) between the two stations in a two station thread has been already demonstrated. The aim here is to develop two ion transporter systems with different transport mechanisms. The 'chloride ion' transporter will have an "antiport" mechanism of transport. In this case the macrocycle binds the chloride and transport it across the bilayer, while simultaneously another substrate is transported in the opposite direction (Figure 11). In this system only the exchange of ions is achieved, not a net transport. In contrast, the 'chloride salt' transporter will have a "symport" mechanism (or co-transport): a functionalised macrocycle crown ether in this case binds two substrates and

transports them together across the membrane together (Figure 11). Partition of the neutral complex into the membrane should be energetically favourable.

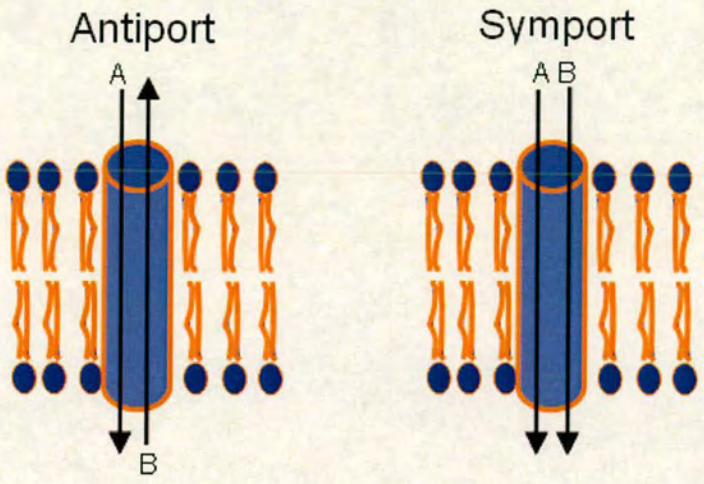


Figure 11. Antiport and Symport mechanisms.

To be compatible with a membrane environment the thread must have the following features:

- a long hydrophobic spacer
- two hydrophilic stoppers or head groups

The thread belongs to the family of "bolaamphiphile" compounds.[33][34, 35] A schematic diagram of the required structural features of the bolaamphiphile thread is shown in Figure 12.

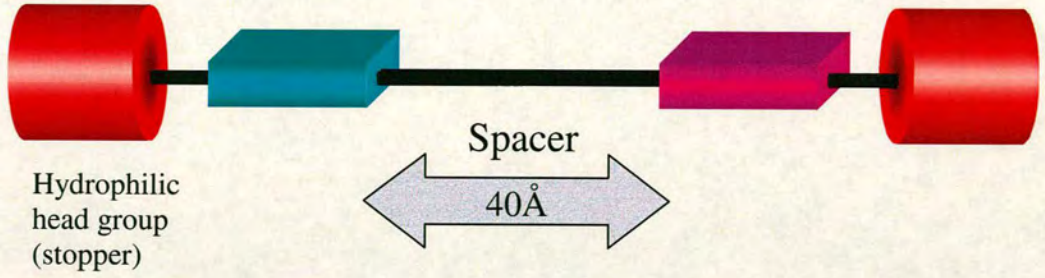


Figure 12. A schematic diagram of the general structure of a thread membrane's compatible.

The structures of the designed membrane spanning [2]rotaxanes are depicted in figures 13 (for the "antiport" system) and 14 (for the "symport" system).

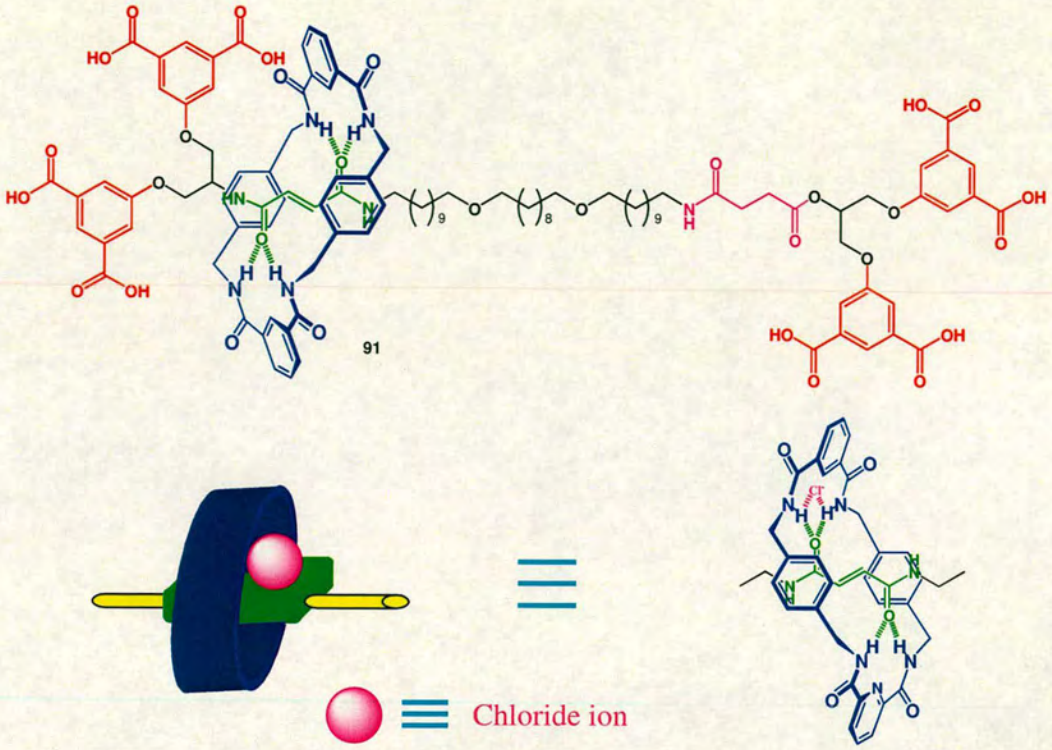


Figure 13. Proposed structure of a membrane spanning [2]rotaxane based on an "antiport" mechanism of transport. (red): stoppers; (bright green): fumaramide/maleamide station; (pink): succinamide-ester station; (blue): tetraamide macrocycle; (pink): chloride ion bound to the macrocycle.

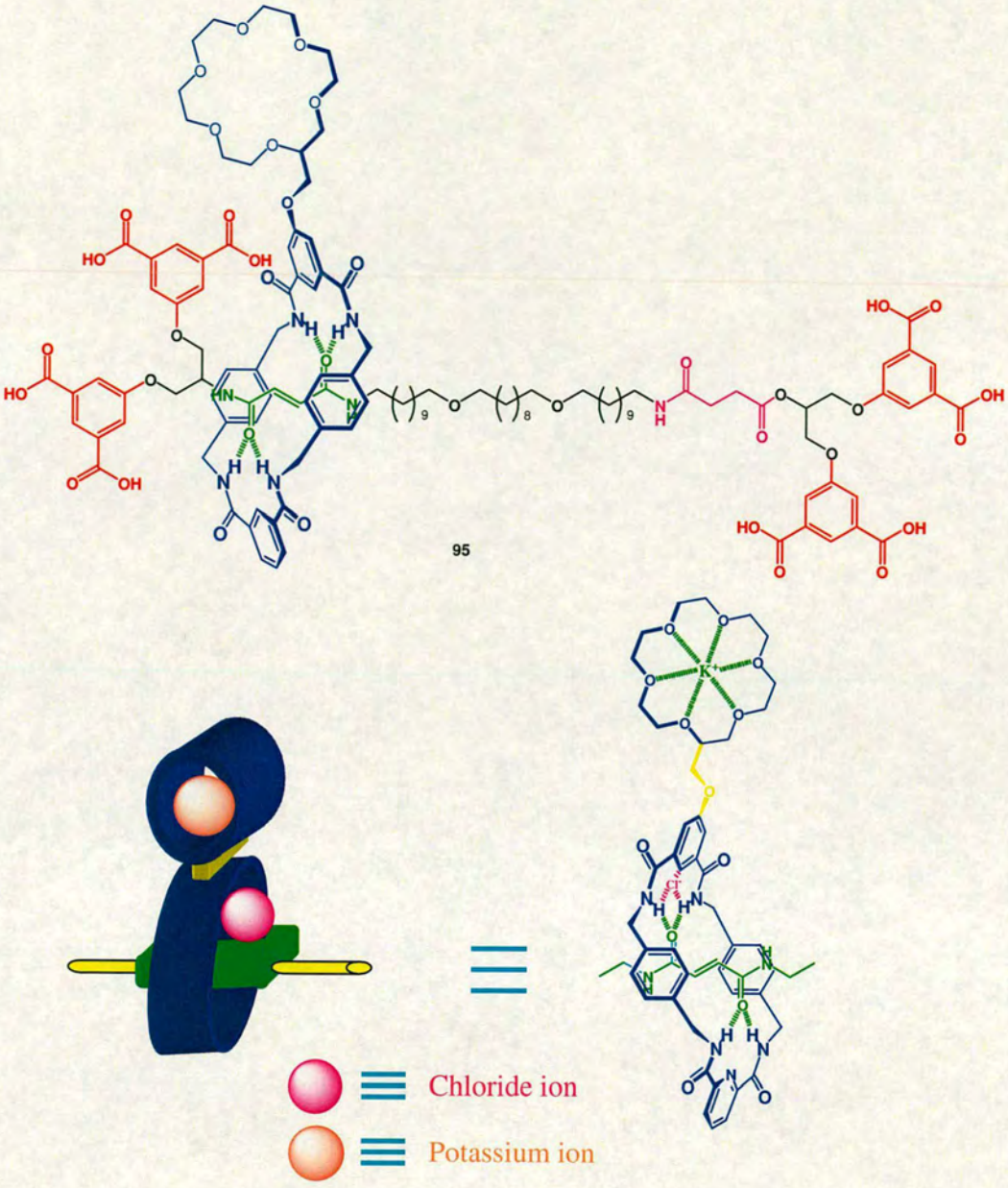


Figure 14. Proposed structure of a membrane spanning [2]rotaxane based on a "symport" mechanism of transport. (red): stoppers; (bright green): fumaramide/maleamide station; (pink): succinamide-ester station; (blue): tetraamide macrocycle functionalized with a 18-crown-ether; (pink and orange): chloride and potassium ions bound to the macrocycle.

3.4.1 Design of the thread (spacer).

While working on channel compounds, Fyles and coworkers[36] reported that transport activity is controlled by certain structural variables. The most active materials tend to have hydrophilic head groups, a balance of hydrophobic and lipophilic groups in the wall unit, and an overall length compatible with the bilayer thickness. The two hydrophilic head groups are essential to provide an amphiphilic characteristic and to assist in the desired transmembrane orientation of the molecule. Furthermore, there should be cooperative interaction between; a) the polar head groups in contact with the aqueous phase, and b) hydrophobic contact between the thread and the lipids of the bilayer[27] The hydrophobic chain of such bolaamphiphile compounds permits them to bridge typical membranes, anchoring their head groups at both outer and inner aqueous/lipid interfaces.[37] Among the most challenging features of these molecules is the question of transporter size. The active structures of synthetic transporters, while being rather small compared to natural systems, are quite large synthetic targets for standard synthetic chemistry. They need to be able to span a 40Å (4 nm) thick bilayer, which corresponds to a linear chain of approximately 30-36 atoms of carbon.[27, 34-49] The spacer must have a length of about 40Å with a hydrophobic character in order to be compatible with the alkyl chain of the liposomes. The two binding stations of the thread must have properties similar to a glyceryl unit, not charged like the stoppers but with a dielectric constant higher than the hydrocarbon portion of the spacer and lower than the one at the interface between water and hydrophilic stoppers.

3.4.2 The Stopper.

The choice of the charged head group stopper is essential for reasons outlined above. It is anticipated that negative charges at both ends of the molecule would favour a stretched conformation[50] and also they should increase the affinity for water. The use of one or more carboxylic groups, deprotonated at the physiological pH, is typical for incorporating charged groups in general artificial systems proposed in the past twenty years.[27, 34, 40, 50-53] The head groups have a second role as stoppers to prevent the dethreading of the macrocycle. In the present case we have replaced the

ubiquitous diphenyl stopper often employed in Leigh's systems[32] with a bis 5-hydroxy isophthalic acid (ISA) moiety (Figure 15). The use of ISAs as amphiphiles has been explored by several groups in various chemical environments and have been shown to form well-ordered structures.[54-59] One of the main reasons for this interest in these groups is due to their propensity to form aggregates involving alkyl group interpenetration in a bilayer like structure.[60]

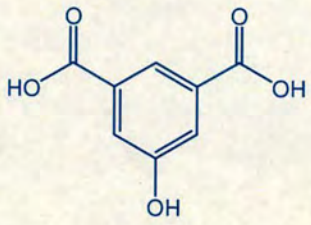


Figure 15. Structure of 5-ISA.

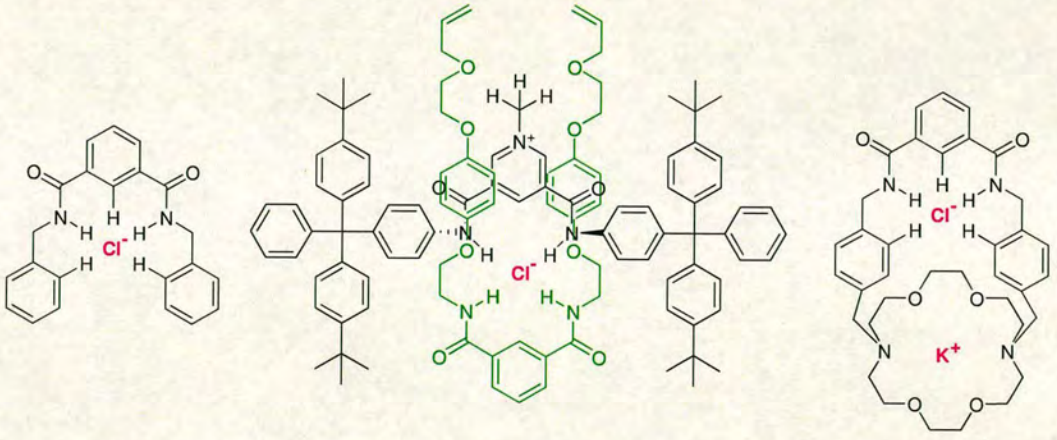
The N-alkoxy isophthalic acid groups help orient the rotaxane in the bilayer membrane by providing a dianionic head group, allowing a sufficiently large structure to span the whole membrane. The presence of this head group at the membrane-solution interface may also assist the cation entry into a channel.[61]

3.4.3. The macrocycle.

The macrocycle of our membrane spanning rotaxane has the role of a carrier that associates with a particular agent and ultimately transports it inside or outside the cell. Obviously, the structure of this group will strongly influence the kind of transport achieved. The first system we deal with involves transport of chloride ions across the membrane, a system that has proven to be a formidable challenge.[62] The macrocycle in this case is based on 2 isophthalamide moieties having amide bonds that adopt a *syn-syn* conformation. Isophthalimides have shown good receptor properties for chloride anions in non competitive solvents. Crabtree and coworkers[63, 64] have studied the properties of isophthalamide ligands and believe that the most powerful binding occurs when the amide bonds are in *syn-syn* conformation (the most energetically unfavourable) (Figure 16, compound **54**). Beer and Wisner[65-67]

reported the formation of interlocked architectures formed by isophthalamide binding of halide ions while templating the formation of [2]pseudorotaxanes (Figure 16, compound **55**). There are also a few examples of chloride ion transporters; the most interesting of these are Smith's cholapods[68] based on steroid receptors and Davis' calix[4]arene tetrabutylamide.[69]

The second system of interest is the chloride salt transporter, also bears a crown ether bound to the tetraamide macrocycle. Binding of cations by crown ethers have been extensively studied in the past 40 years[70] and many examples of their use in ion transport exist.[71-76] Cations such as K^+ and Na^+ will associate these types of crown ethers while the chloride anion (present as counter anion) will likely bind the isophthalamide system as seen above. Smith and coworkers[77, 78] have extensively studied salt transport by ditopic salt binding receptors, where one molecule binds two opposite charged species (Figure 16, compound **56**) .



54 **55** **56**

Figure 16. (a) Crabtree's isophthalamide ligand **54**, (b) Beer and Wisner's templated formation of rotaxanes and (c) Smith's ditopic salt **56**.

3.5 Spanning the membrane and transport.

The spanning process can be subdivided in two key steps:

- diffusion of the membrane spanning rotaxane
- insertion into the bilayer

The diffusion of the [2]rotaxane does not imply any specific interaction. The insertion step of a U-shaped folded state is a logical possibility, followed by penetration of one of the head groups to generate the transmembrane state. An alternative would be a threading insertion to generate the trans-membrane state.

The transport process

After photochemical isomerization of the fumaramide station, the ions which are bound to the macrocycle are carried through the membrane. This is the essence of the entire project – controllable ion transport across a lipid membrane. Inside the vesicle the aqueous environment promotes the release of the ion from the macrocycle. Due to the symmetry of the rotaxanes (since both stoppers are identical) they will orient themselves equally in the two possible ways. The back and forth movement between the two stations allows the macrocycle of the membrane spanning rotaxane to cross the entire bilayer in a reversible process. The aim is to establish transport of ions between both aqueous phases. Figure 17 shows how a membrane spanning rotaxane based on photo active stations works.

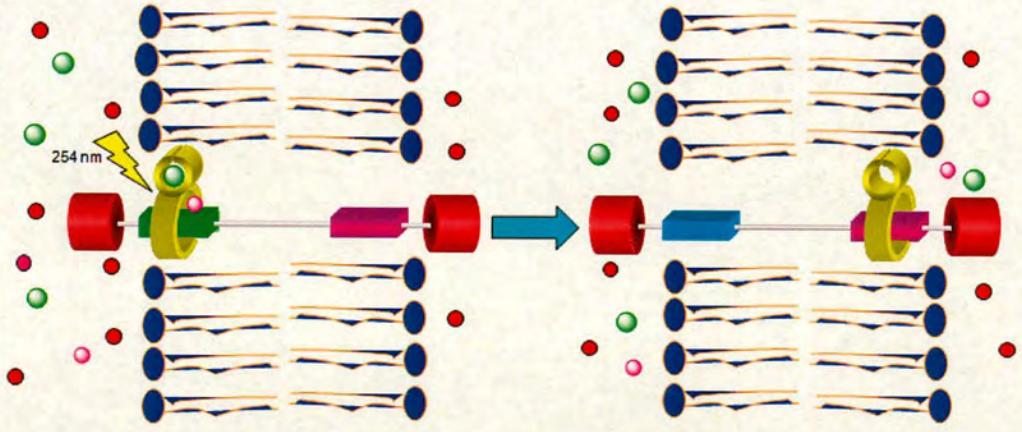
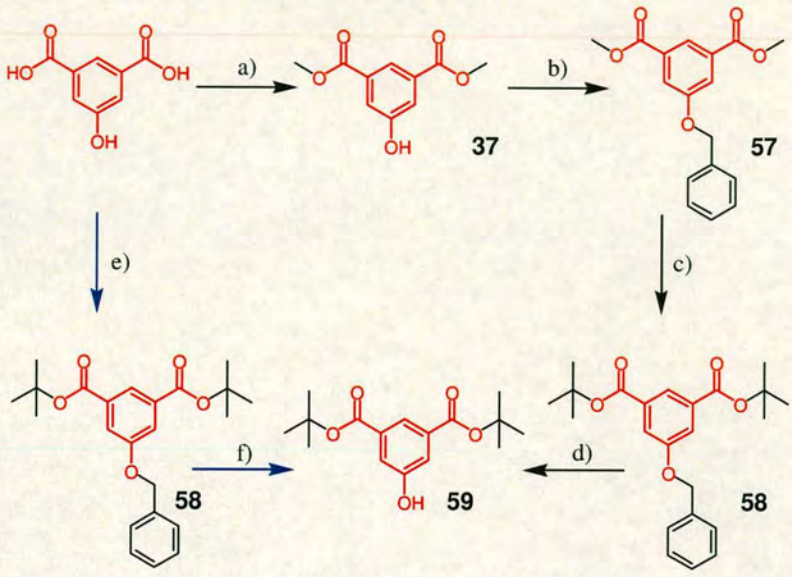


Figure 17. Proposed motion of a membrane spanning [2]rotaxane in membrane.

3.6 The Synthesis.

3.6.1 Synthesis of the end group

The synthesis of end group **59** is shown in scheme 1.



Scheme 1. Synthesis of the end group **59**. Reagents and yields: a) H_2SO_4, MeOH, reflux, 91%; b) BnBr, K_2CO_3, 2-butanone, reflux, 76%; c) *t*BuOK, *t*BuOAc, THF, 55%; d) Pd/C, H_2, MeOH, 99%; e) $SOCl_2$, $(COCl)_2$, (9;1), reflux, then *t*BuOK, THF, then BnBr, 2-butanone, reflux, 69%; f) Pd/C, H_2, MeOH, 99%.

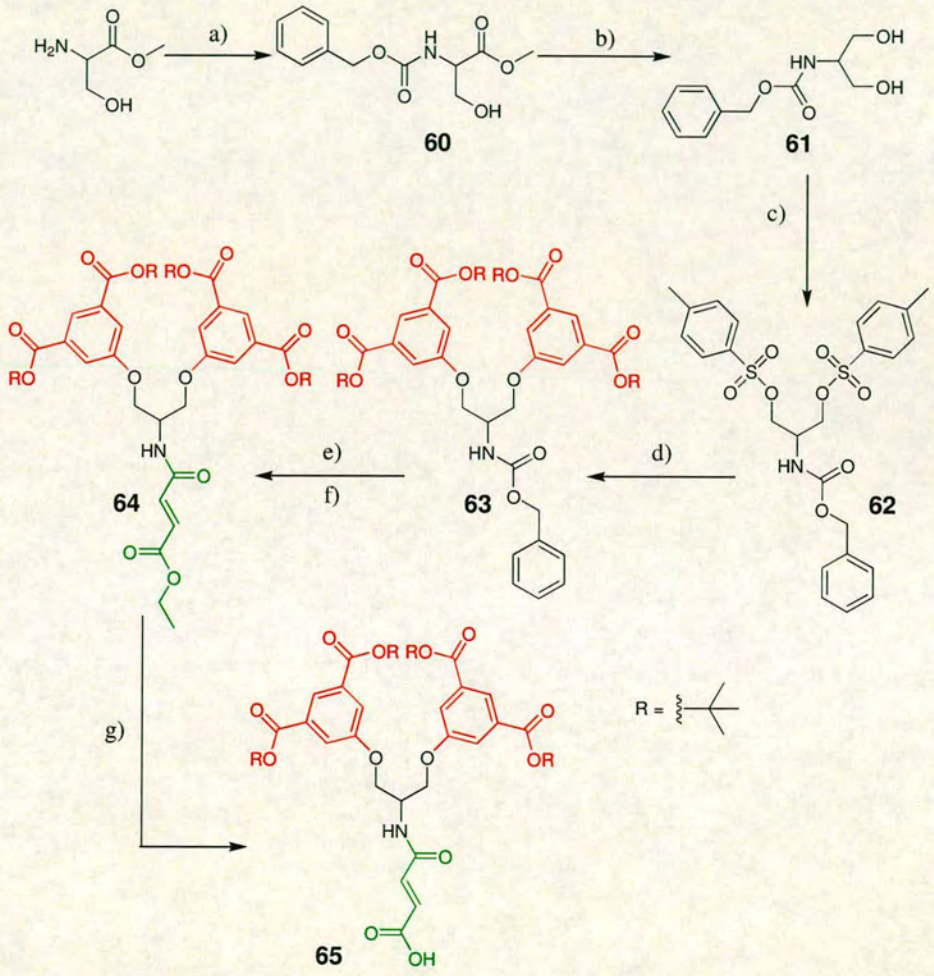
Two approaches were employed to synthesize **59**. The first one (route **a-d**) was via "ester interchange reaction" in which a methyl or ethyl ester can be converted to a correspondent *tert*-butyl ester using potassium *tert*-butoxide in THF as the catalyst and *tert*-butyl acetate as solvent.[79] This reaction works well with an aromatic ester but not so with **37** because of competing deprotonation processes between the free OH group and potassium *tert*-butoxide. Compound **37** was prepared using Fisher's esterification (refluxing the di-carboxylic acid with MeOH in the presence of sulfuric acid as catalyst) in good 91% yield (Scheme 1, step a). The hydroxyl was protected with a benzyl group, by refluxing **37** with benzyl bromide in 2-butanone using K_2CO_3 as base, to give **57** in a moderate yield of 76% (Scheme 1, step b). Compound **58**, was obtained via ester interchange from **57** in 55% yield (15% of mono-substituted product was also isolated) (Scheme 1, step c). Finally removal of the

benzyl group was achieved using hydrogen, Pd/C as catalyst, and MeOH as solvent affording **59** in quantitative yield (Scheme 1, step d). An alternative shorter method was also developed (route **e-f**).

3.6.2 Synthesis of the Fumaric station.

The molecule at the core of the synthesis of the fumaric station is Cbz-Serinol (**61**). Scheme 2 shows the synthesis of the fumaric station. This molecule has two OH functional groups and a protected NH group. The two primary alcohols are required for the subsequent attachment of the bulky stopper groups and the NH forms part of the fumaric station. As discussed above the fumaric amide-amide group was chosen as a first candidate for a station on the thread. Compound **61** was prepared as follows. Serine methyl ester was protected with a Cbz group, by reaction with Cbz-succinimmide giving **60** in very good yield (Scheme 1, step a) then compound **60** was reduced with $LiBH_4$ in THF using an equimolar amount of MeOH to enhance the reactivity of the lithium salt (Scheme 2, step b); Cbz-Serinol **61** was then obtained after flash chromatography of the crude product in good yield (77%). Attachment of **59** to Cbz-Serinol **61** was firstly attempted using a Mitsunobu reaction with Ph_3P/DIAD in THF at 0 °C. Several attempts of this reaction lead to a low yield of **63** as well as mono addition products and several other byproducts. Kramer and coworkers[80] and Vederas and coworkers[81] observed that a free NH proton may react during the Mitsunobu reaction. The problem was avoided by choosing a different path for the di-addition of the end group. The two primary alcohols were activated with a *p*-toluene sulfonyl group, using *p*-toluene sulfonyl chloride and pyridine in $CHCl_3$, to give **62** in reasonable yield (60%) (Scheme 2, step c). The di-addition reaction was performed by reacting **62** with **59** in refluxing CH_3CN employing K_2CO_3 as a base (Scheme 2, step d). The reaction was complete within 6 hours, furnishing **63** in a 45% yield. No improvements were observed using 2-butanone and refluxing the reaction for a longer time. Removal of the Cbz group of **63** in MeOH under catalytic hydrogenation (using Pd/C as catalyst) gave the free amine in quantitative yield. Addition of the fumaric moiety yielded **64** (Scheme 2, step e and f). This was achieved by converting the carboxylic acid of the correspondent fumaric acid mono-ethyl ester to a more reactive chloride using oxalyl

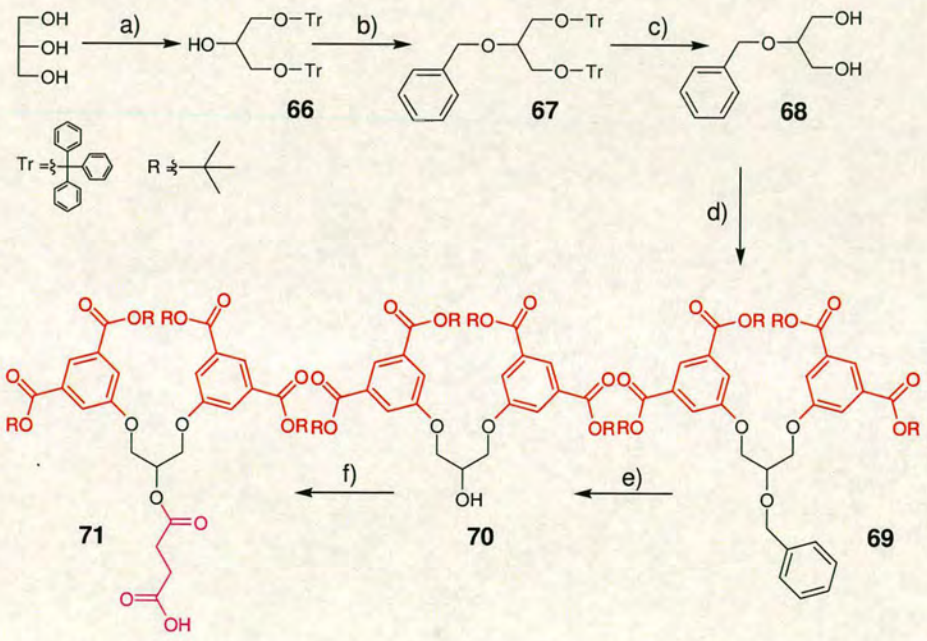
chloride adding a drop of DMF as catalyst in CH_2Cl_2. The yield was unexpectedly low possibly due to steric hindrance of the two bulky stoppers. The ethyl protecting group was removed from **64** with an equimolar solution of NaOH (in THF and water) yielding **65** (95% yield) (Scheme 2, step g). No loss of *t*Bu groups was observed at this stage.



Scheme 2. Synthesis of the fumaric station **65**. Reagents and yields: a) Nα-(Benzyloxycarbonyloxy) Succinimide, Et_3N, $CHCl_3$, 96%; b) $LiBH_4$, THF, MeOH, 77%; c) *p*-tolunenesulfonyl chloride, pyridine, CH_2Cl_2, 60%; d) K_2CO_3, **59**, 2-butanone, reflux, 45%; e) Pd/C, H_2, quantitative; f) fumaric acid monoethyl ester chloride, Et_3N , CH_2Cl_2, 40%.

3.6.3 Synthesis of the succinic station.

The key intermediate molecule for the synthesis of the succinic station is 2-O-benzyl glycerol **68**. Scheme 3 shows the synthesis of the succinic station. This fragment has three OH functional groups: the two primary alcohols are free and the secondary one is protected with a benzyl group. The two primary alcohols were needed for the attachment of the bulky end groups as described in the proceeding section, and the secondary was needed for furnishing the succinic station. As discussed above the succinic amide-ester is a suitable candidate for a second station of the asymmetric thread.



Scheme 3. Synthesis of the succinic station **71**. Reagents and yields: a) chlorotriphenylmethane, pyridine, 97%; b) BnCl, KOH, toluene, reflux, 81%; c) HOAc (80%), 100°C, 30 min, 51%; d) **59**, Ph_3P, DIAD, THF, 45%; e) Pd/C, H_2, HOAc, 75%; f) succinic anhydride, Et_3N, CH_2Cl_2, 90%.

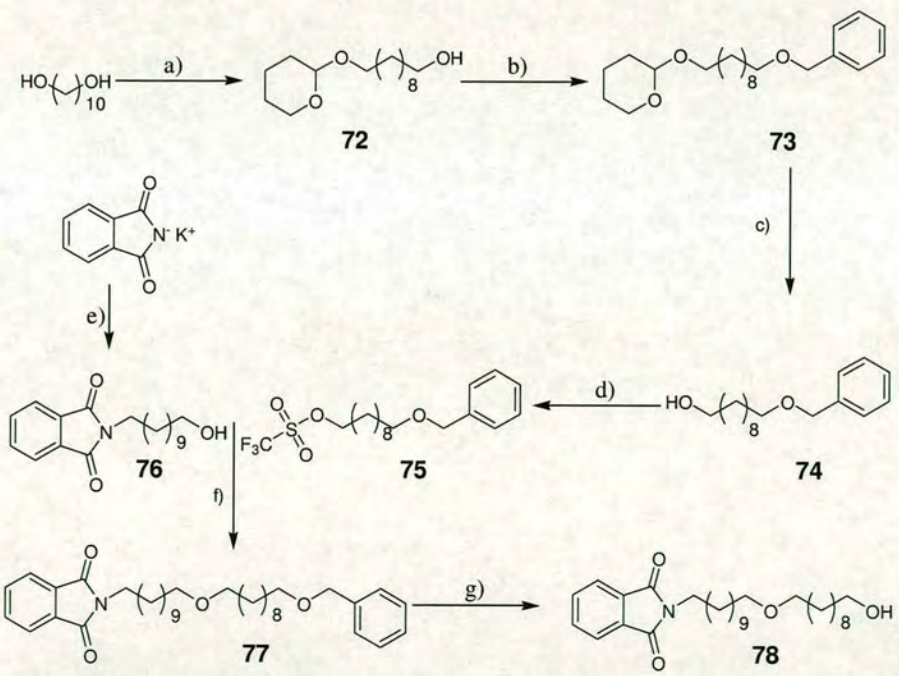
The 2-O-benzyl glycerol moiety was prepared following the procedure described by Krebs et al.[82] Glycerol is selectively protected in the 1 and 3 positions by two bulky trityl groups using chlorotriphenylmethane and pyridine as solvent yielding **66** essentially quantitatively (Scheme 3, step a). Then the secondary alcohol is subsequently protected with a benzyl group by refluxing benzyl chloride in toluene

using KOH as base. Compound **67** was obtained in 81% yield (Scheme 3, step b). The removal of the two trityl groups was achieved in HOAc 80% at 100 °C and, after a purification by flash chromatography compound **68** was obtained in 51% yield (Scheme 3, step c). Alkylation of the two primary alcohols and attachment of **59** is performed as before via Mitsunobu reaction (using Ph_3P/DIAD in THF at 0 °C), providing **69** in moderate yield (45%) (Scheme 3, step d). The mono addition product has been isolated in 25% yield. Surprisingly, our first attempt at activating the diol with two *p*-toluene sulfonyl groups and subsequent alkylation with **59** under refluxing 2-butanone (using K_2CO_3 as base) gave the di-addition product in 5% yield. We recovered almost all the starting material using the same conditions employed for the fumaric station and no improvements were observed using DMF at higher temperatures (120 °C) or refluxing the reaction using 2–butanone for a longer time. Removal of the benzyl group by catalytic hydrogenation carried out in neat HOAc obtaining **70** in 75% yield (Scheme 3, step e). Several previous attempts in various solvents (EtOH, THF) tried without success. Surprisingly, the *t*BuO group showed good stability under these conditions, only minimal decomposition was observed during the removal of the benzyl group. Finally, addition of succinic anhydride in the presence of catalytic amount of Et_3N to **70** in CH_2Cl_2 produced succinic station **71** in good yield (90%) and was used without any further purification (Scheme 3, step f).

3.6.4 Synthesis of the spacer.

It is important that the spacer have two NH_2 groups, one on either end, and that they be protected with different protecting groups to allow selective removal of them for the subsequent coupling reactions with the two different stations (*vide supra*). The spacer has a 32 carbon length connected by two ether bonds. It's divided into three fragments; two 11 atom carbon chains and one of 10 carbon atoms long connected by ether linkages. One end is protected with a phthalimide group and the other with a Cbz group. The synthesis of the first two fragments is shown in scheme 4. The fragments of the spacer were coupled together by SN_2 reaction following Springer[83] and Anderson[84] in which the alcohol was partially deprotonated in refluxing CH_2Cl_2 using 1,8-bis(dimethylamino)naphthalene (Proton Sponge) as a base and

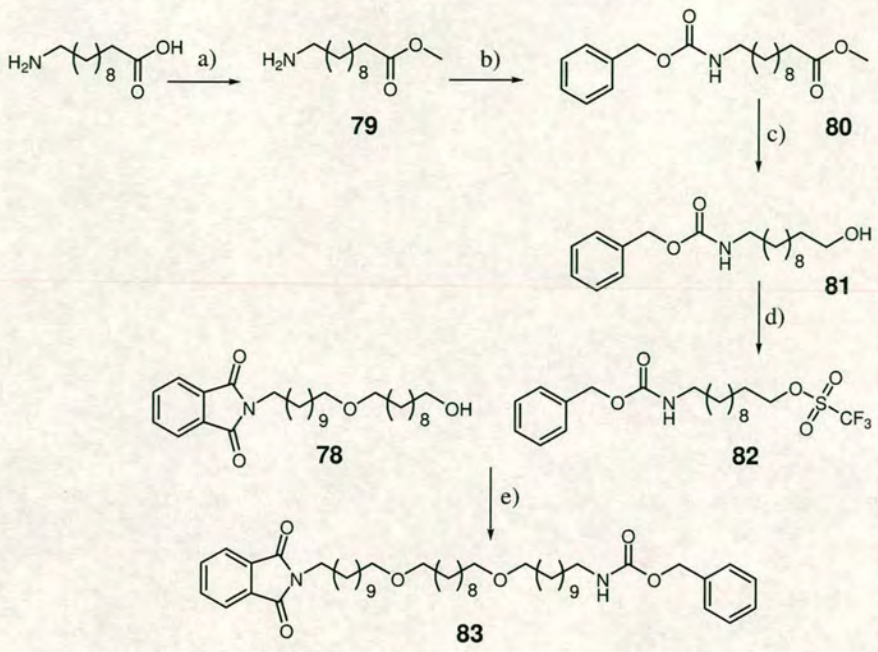
subsequently using alkyl triflate for its beneficial leaving group properties. The base was chosen for its inertness in inducing few side reactions. Compound **72** was prepared from the 1,10-decanediol by selective mono-protection, catalyzed by hydrogen ion in the water layer in contact with DCP-hexane-DMF layer in 70% yield (Scheme 4, step a). We observed that **72** is not suitable for a reaction where trifluoroacetic anhydride is involved, therefore, we changed the protecting group of the alcohol from THP to benzyl to improve its stability in the acidic environment. Reaction of **72** in DMF using NaH as base and benzyl bromide in the presence of catalytic amount of tetrabutyl ammonium iodide furnished **73** in 79% yield (Scheme 4, step b). Removal of the THP group was accomplished in ethanol using PPTS at 70°C, and yielded **74** in 81% yield (Scheme 4, step c). The primary alcohol, **74**, was activated with trifluoromethane sulfonic anhydride and pyridine in CH_2Cl_2 at room temperature to give **75** in 85% yield (Scheme 4, step d).



Scheme 4. Synthesis of the first fragment of the spacer **78**. Reagents and yields: a) DHP/Hexane (97:1), DMSO, $NaHSO_4$ (aq), 70%; b) BnBr, NaH, TBAI, DMF, 76%; c) PPTS, EtOH, 55 °C, 81%; d) Tf_2O, pyridine, CH_2Cl_2, 85%; e) bromoundecanol, 80 °C, DMF, 95%; f) **76**, **75**, Proton Sponge, CH_2Cl_2, 88%; g) Pd/C, H_2, HOAc, 75%.

The alkylation reaction between alcohol **76** and alkyl triflate **75** with base in refluxing CH_2Cl_2 afforded **77** in excellent 88% yield (Scheme 4, step f). Removal of the benzyl group using hydrogen and Pd/C was surprisingly unsuccessful. Here also, as with the removal of the benzyl group for the succinic station, neat HOAc was employed. Unfortunately, signs of phthalimide decomposition were observed. Removal of the benzyl group afforded free alcohol **78** in good yield (80%) (Scheme 4, step g).

Compound **79** was obtained from the corrisponding carboxylic acid in refluxing methanol using thionyl chloride and was isolated in quantitative yield (scheme 5, step a). The free amine was protected with a Cbz group using Nα-(Benzyloxycarbonyloxy) Succinimide and Et_3N in $CHCl_3$ to give **80** in 90% yield (Scheme 5, step b). The methyl ester was then reduced to the primary alcohol **81** using $LiBH_4$ and MeOH in THF in 77% yield (Scheme 5, step c). A first attempt at OH activation of **81** with trifluoromethane sulfonic anhydride and pyridine in CH_2Cl_2 afforded poor yields. The Cbz group was cleaved during the triflate activation of the alcohol when the reaction was performed at room temperature. Lowering the temperature to –78 °C allowed the activation of the last fragment without any loss of Cbz protecting group. **82** was obtained in 70% yield (Scheme 5, step d). The reaction between alkoxide **78**, and alkyl triflate **82** using proton sponge as base in refluxing CH_2Cl_2 furnished spacer **83** in 60% yield.



Scheme 5. Synthesis of the spacer **83**. Reagents and yields: a) $SOCl_2$, MeOH, reflux, 99%; b) Nα-(Benzyloxycarbonyloxy) Succinimide, Et_3N, $CHCl_3$, 90%; c) $LiBH_4$, THF, MeOH, 77%; d) Tf_2O, pyridine, CH_2Cl_2, -78 °C, 70%; e) 24, 28, proton sponge, CH_2Cl_2, reflux, 60%.

3.6.5. Synthesis of the thread

For convenience, thread **86** will be described in three separate parts as shown by scheme 6:

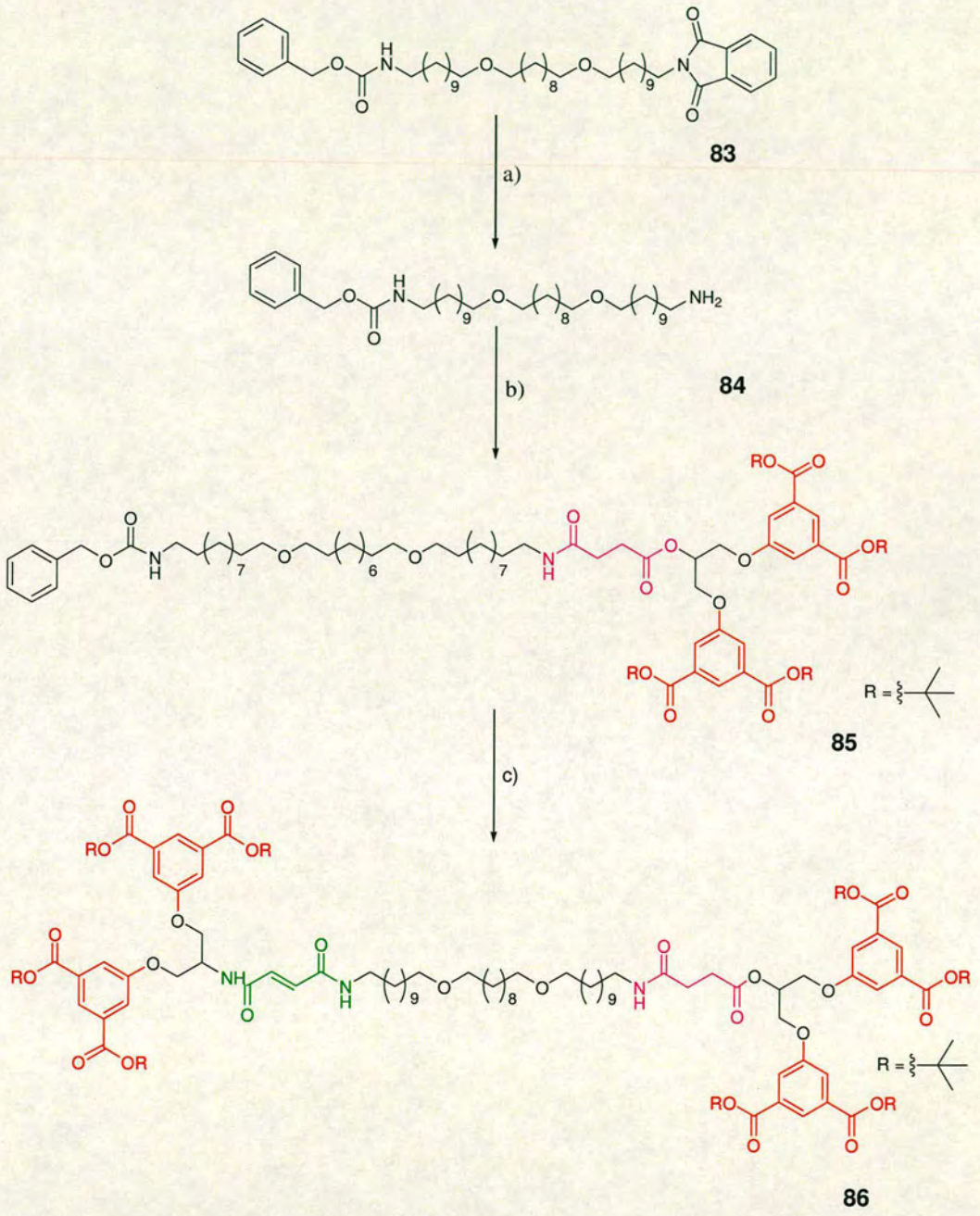
i) the spacer (**83**)

ii) the fumaric station (**65**)

iii) the succinic station (**71**)

The spacer contains two ether links, one at either end, and also two primary amines protected with different protecting groups (Cbz and isophthalamide). Removal of the isophthalamide protecting group using hydrazine in refluxing ethanol allows one to obtain the mono-protected spacer **84** with a good yield with minimal loss of Cbz (Scheme 6, step a). The succinic station **71** and mono-protected spacer **84** were coupled together using EDCI and HOBt yielding fragment **85** in 80% yield (Scheme 6, step b). Removal of the Cbz group via catalytic hydrogenation in methanol in compound **85** produced the free amine necessary for the further coupling with the

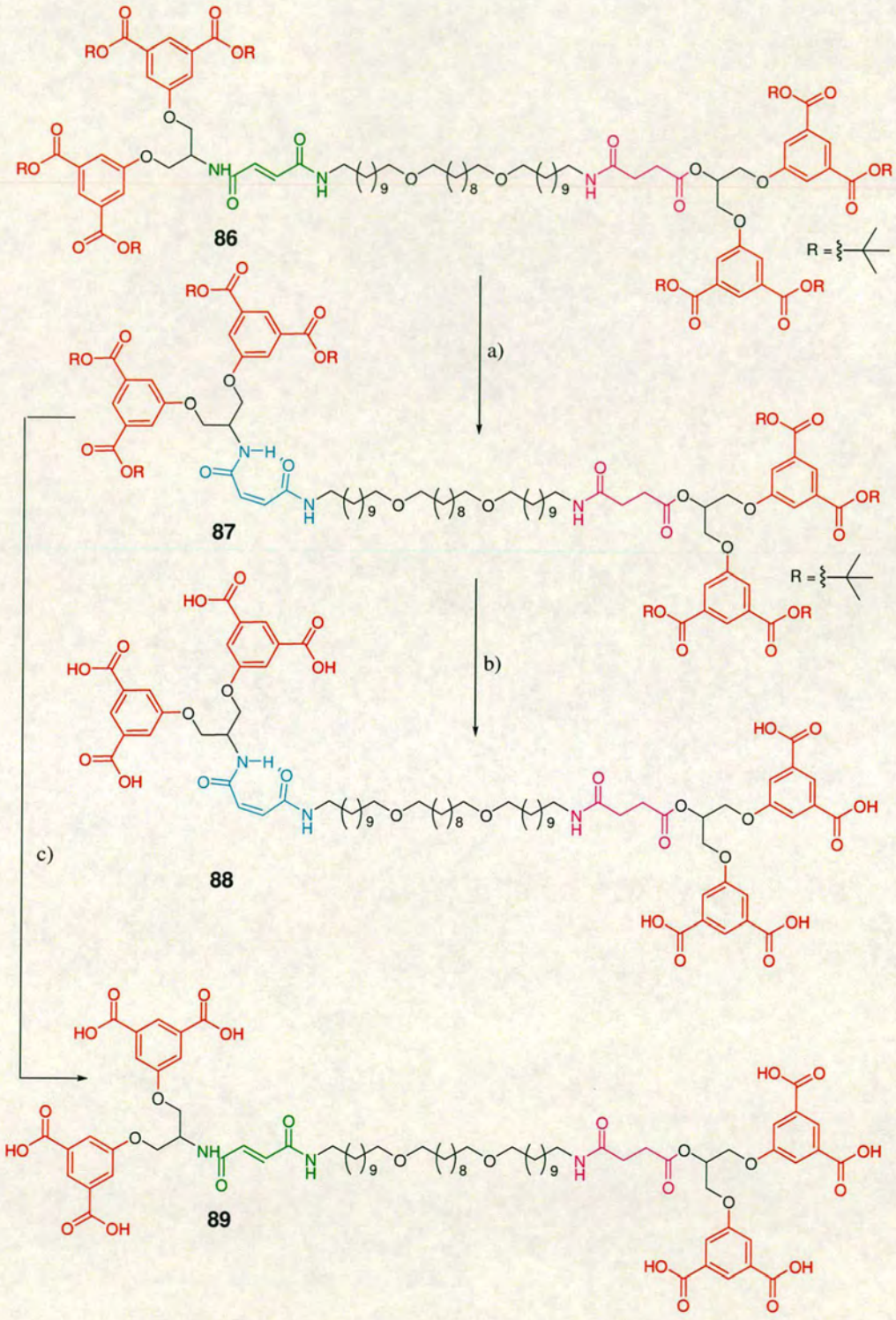
fumaric station **65**. Thread **86** was synthesized from **65** and **85** (Cbz deprotected) using EDCI and HOBt in 60% yield (Scheme 6, step c).



83 **84** **85** **86**

Scheme 6. Synthesis of the thread. Reagents and yields: a) N_2H_4, EtOH, reflux, 96%; b) succinic station **71**, EDCI, HOBt, Et_3N, CH_2Cl_2, 77%; c) Pd/C, H_2, MeOH, quantitative, then fumaric station **65**, EDCI, HOBt, Et_3N, CH_2Cl_2, 60%.

3.6.6 Synthesis of the *cis* and *trans* deprotected threads.



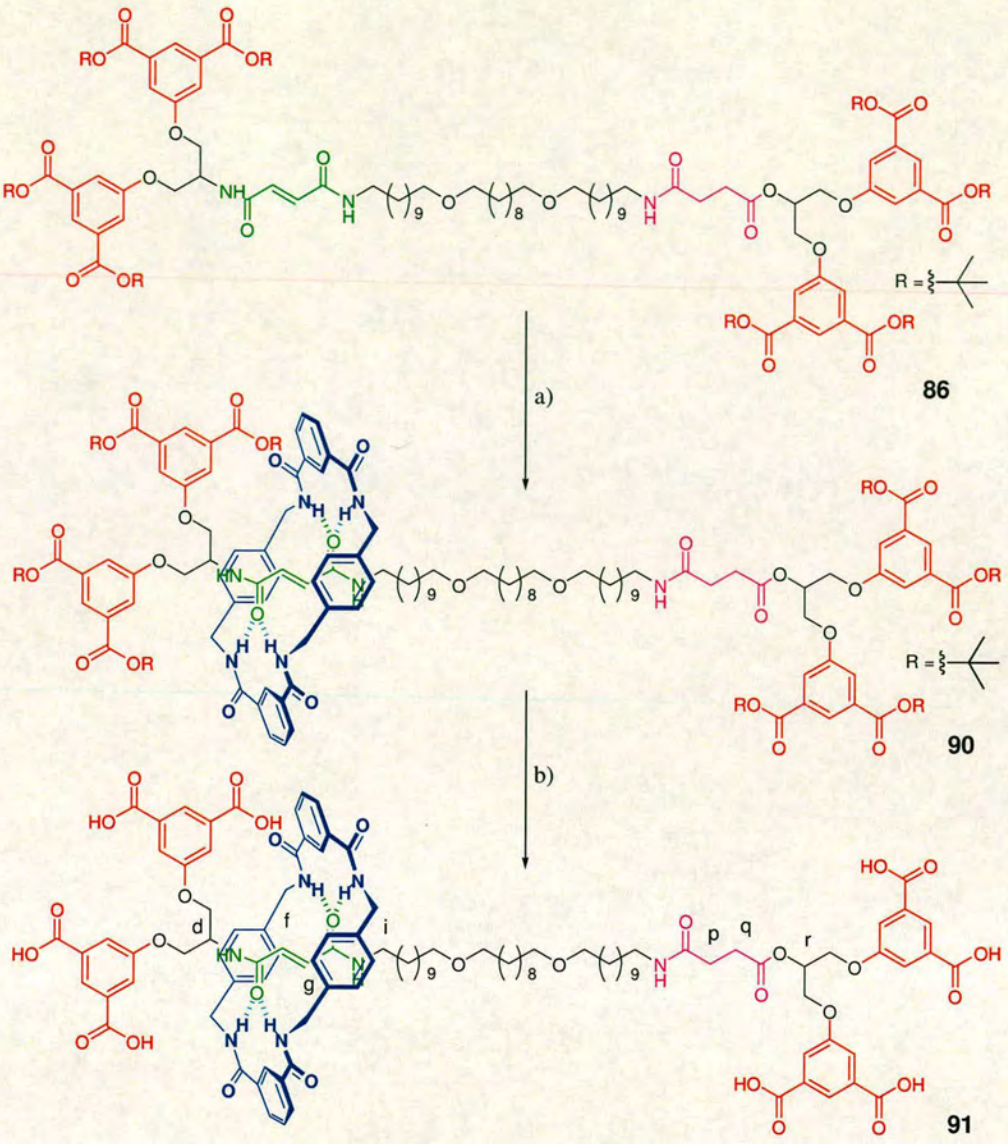
Scheme 7. Synthesis of the *cis* **88** and *trans* threads **89**. Reagents and yields: a) 254 nm, CH_2Cl_2, 20 min, 30%; b) CH_2Cl_2/TFA (9:1), 99%; c) CH_2Cl_2/TFA (9:1), 99%.

Cis *t*Bu protected thread **87** was obtained by irradiation of the *trans* *t*Bu protected thread **86** at 254 nm (Scheme 7, step a) and the two corresponding deprotected *trans* and *cis* threads **89** and **88** were obtained by removal of the *t*Bu protecting groups using TFA in high yields (Scheme 7, step b). Scheme 7 shows the synthesis of the threads.

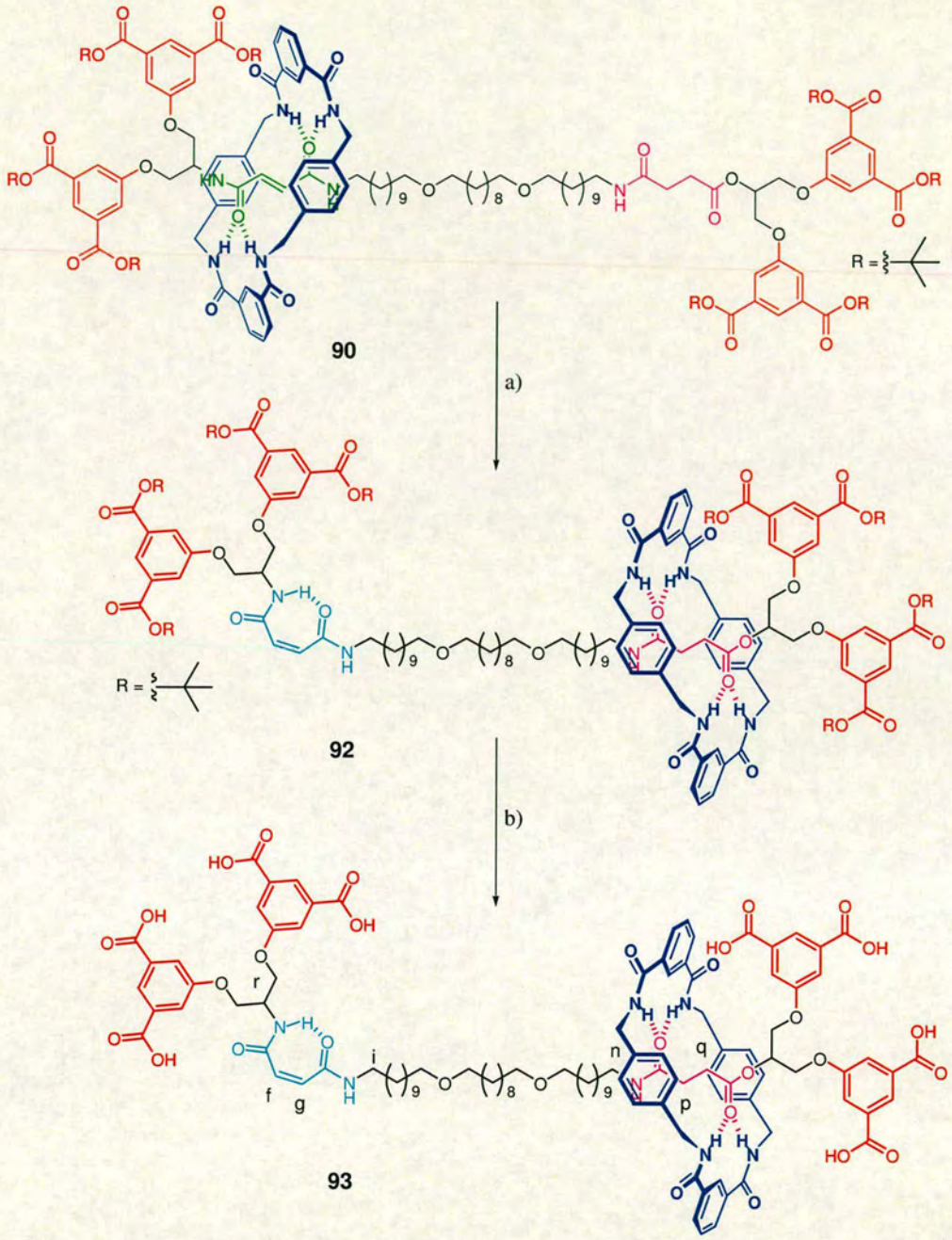
3.7 Synthesis of the membrane spanning rotaxanes.

3.7.1 Synthesis of the *trans* and *cis* 'chloride ion' membrane spanning rotaxane.

Trans rotaxane *t*Bu protected **90** has been synthesized by a five-component hydrogen bond directed clipping reaction from *trans* thread **86** in high yield by simultaneously reacting xylylene diamine and isophthaloyl dichloride in a stoichiometric syringe pump-driven reaction (Scheme 8, step a). Removal of the *t*Bu protecting groups to obtain compound **91** was accomplished using conditions described above (Scheme 8, step b). *Cis* *t*Bu protected rotaxane **92** was obtained by irradiation of the *trans* *t*Bu protected rotaxane **90** with UV light at 254 nm (Scheme 9, step a). The corresponding deprotected *cis* rotaxane **93** was obtained by removal of the *t*Bu protecting groups of compound **92** with TFA (Scheme 9, step b). Schemes 8 and 9 show the synthesis of the *cis* and *trans* 'chloride ion' membrane spanning rotaxanes.

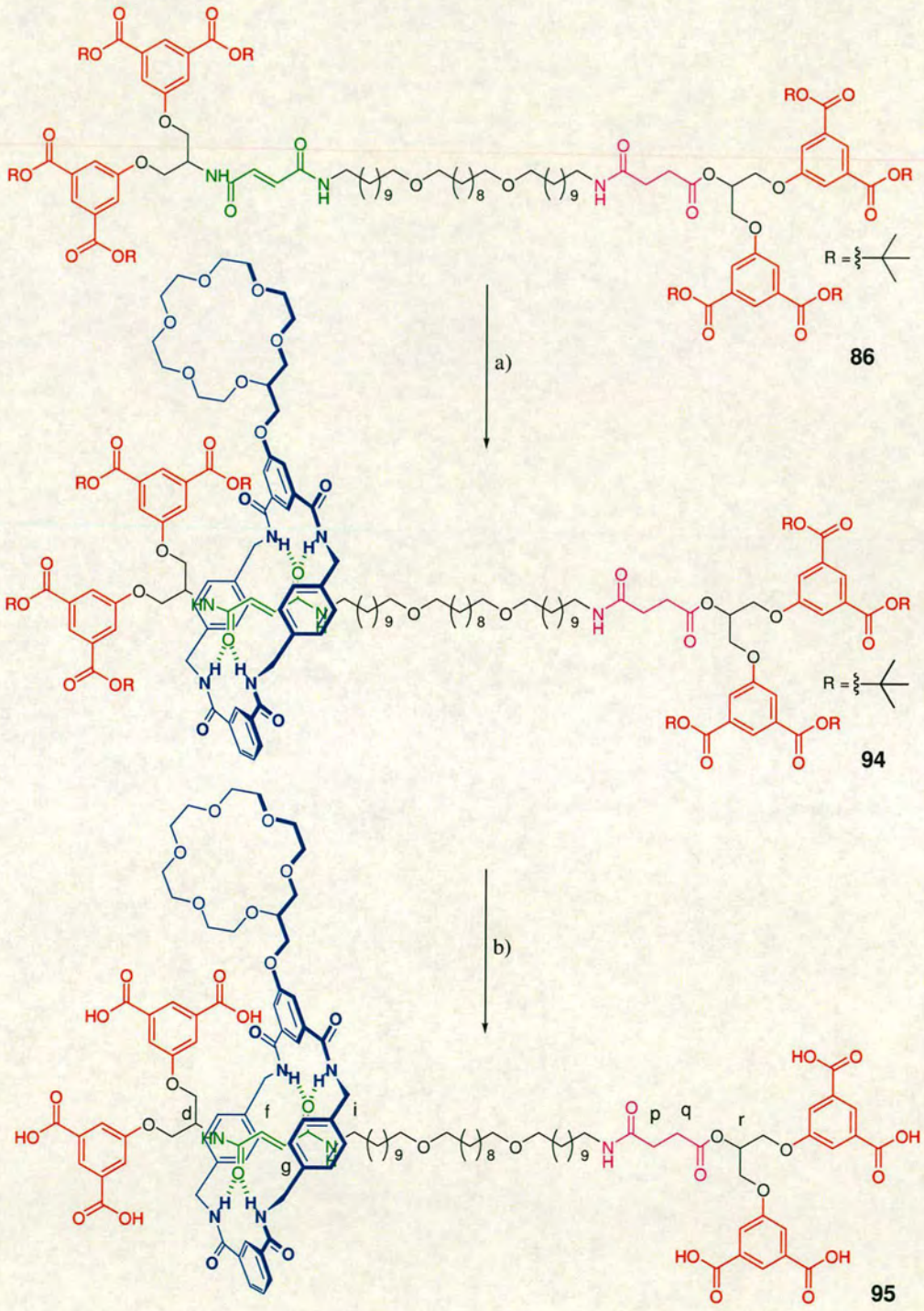


Scheme 8. Synthesis of the *trans* rotaxanes **90** and **91**. Reagents and yields: a) isophthaloyl dichloride, xylylene diamine, Et_3N, $CHCl_3$, 85%; b) CH_2Cl_2/TFA (9:1), 99%.



Scheme 9. Synthesis of the *cis* rotaxanes **92** and **93**. Reagents and yields: a) 254 nm, CH_2Cl_2, 20 min, 35%; b) CH_2Cl_2/TFA (9:1), 99%.

3.7.2 Synthesis of the *trans* chloride salt membrane spanning rotaxane.



Scheme 10. Synthesis of rotaxane **95**. Reagents and yields: a) compounds **44** and **39A** $CHCl_3$, 15%; b) CH_2Cl_2/TFA (9:1), 99%.

Scheme 10 shows the synthesis of the *trans* rotaxane **94** and **95**. Macrocyclization of the ¾-macrocycle bearing and endotopic nitrogen **44** (from Chapter 2) and the correspondent dichloride compound **39A** obtained from **39** (from Chapter 2) around the thread **86** in $CHCl_3$ yielded [2]rotaxane **94** in a 15% yield using 3 equivalents of ¾-macrocycle (Scheme 10, step a). The yield, compared to the system based on a single station proposed in chapter 2, is lower than expected. However, given the complexity of the current system, several reasons for this decrease in yield can be envisioned, for example. There is the possibility of formation of [3]rotaxane since the thread, being quite long, means that there is ample space for the interlocked macrocycle to move away from the fumaric template, allowing another macrocycle to form around this site.[85] Secondly, by its nature, the crown ether is a strong binder and may be competing with other species present in solution, in particular with the protonated amine of the preformed 3/4-macrocycle, and not allowing cyclisation to proceed. Thirdly, the thread is not at all rigid, compared for example to the small fumaric thread, and since the long spacer allows the thread to be more flexible and folding of it could hinder templating reactions. Removal of the *t*Bu protecting groups on compound **94** to obtain compound **95** in high yield was performed by using TFA (Scheme 10 step b).

3.8.1 Characterization: evaluation of shuttling in the 'chloride ion' membrane spanning rotaxane.

In this section we evaluate data from the shuttling studies performed on rotaxanes **91** and **93**. As expected, analysis of the system based on the simpler tetra amide macrocycle proved to be easier than that based on the more complicated crown functionalized macrocycle. The position of the macrocycle may be determined by comparing the chemical shifts of the protons in the rotaxane with those of the corresponding thread due to shielding by the xylylene rings of the encapsulated region of the thread. The spectra of thread **89** and rotaxane **91**, thread **88** and rotaxane **93** in CD_3OD (400 MHz, 298K) are shown in Figure 18 and 19 respectively.

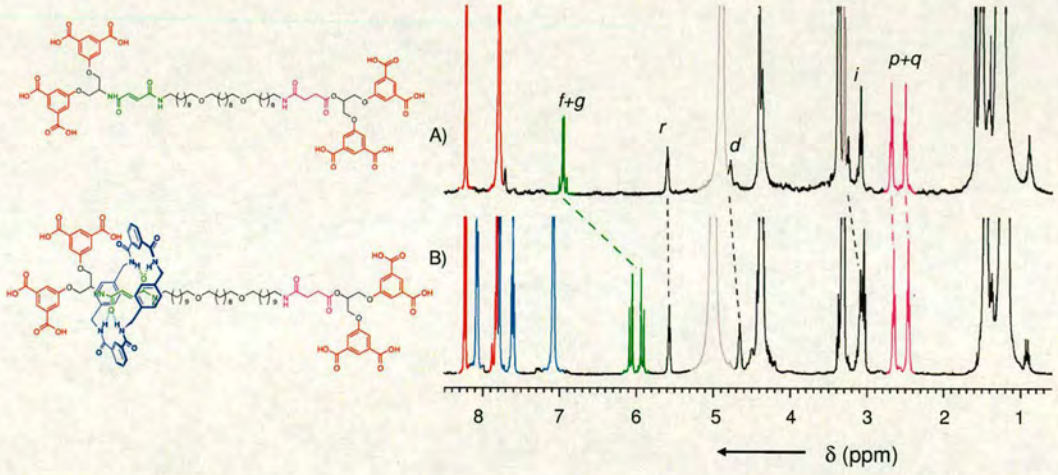


Figure 18. ^1H NMR (400 MHz, 298 K,CD_3OD) spectra of (a) thread **89** and (b) rotaxane **91** (for hydrogen labels refer to scheme 8).

The H_f and H_g protons of the fumaramide group are shielded in the rotaxane compared with the free thread by approximately 1.02 ppm, indicating the likelihood that the macrocycle mainly resides over this portion of the thread. The chemical shifts of the H_p and H_q protons of the Succinic amide-ester group are just slightly shifted upfield by 0.14 ppm. In the maleamide isomer the situation is reversed (Figure 19). Since the H_i proton also shifts slightly, the situation in solution may be one in which there is folding of the rotaxane so that the carbonyl adjacent to H_i also

hydrogen bonds to the macrocycle. This should no longer be a problem in the membrane embedded rotaxane.

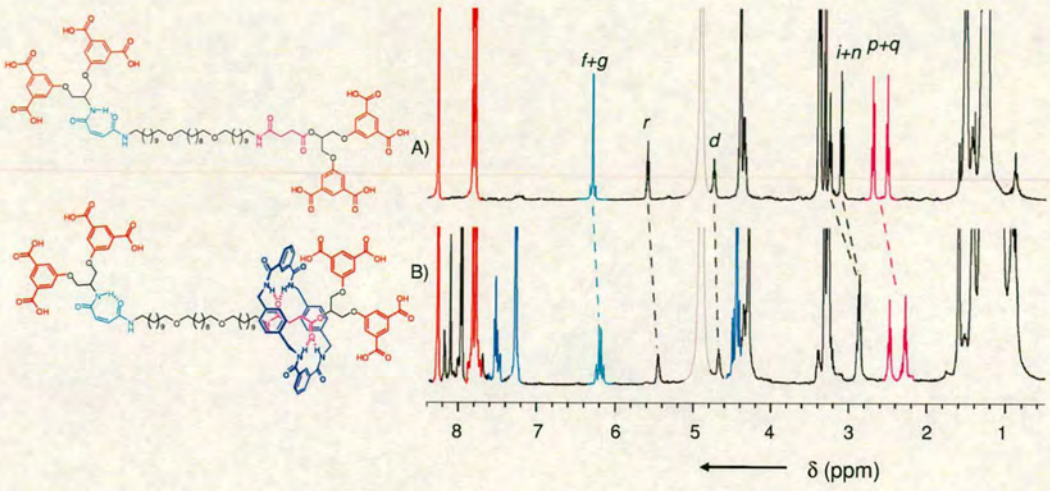


Figure 19. ^{1}H NMR (400 MHz, 298 K, CD_3OD) spectra of (a) thread **88** and (b) rotaxane **93** (for hydrogen labels refer to scheme 9).

The Z-olefins proton H_f and H_g are just slightly shifted upfield by 0.07 ppm in the rotaxane whereas the succinic amide-ester protons H_p and H_q are each shielded by 0.20 ppm in the rotaxane, indicating a predominance of the species having the macrocycle over the succinic functionality.

The NMR spectra of the *trans* thread **89** and rotaxane **91** (Figure 18) are significant. At 298K the occupancy of the fumaramide is greater than 80%. A lower selectivity is observed in the *cis* rotaxane. In comparing similar two station systems reported previously with the present case, several differences are noteworthy:

- This is the first example of a system where the two stations are far apart at 40Å (4 nm) and at room temperature the macrocycle appears to be positioned over the fumaramide station the majority of the time.
- The solubility of the rotaxanes is improved if CD_3OD is used instead of $CDCl_3$, however, this solvent is a stronger hydrogen bond competitor compared to chloroform. The selectivity results obtained in CD_3OD demonstrate the unusually powerful binding of the fumaramide template to the macrocycle. In $CDCl_3$ (with the isomer containing stoppers protected by

tBu groups) the selectivity of the macrocycle for the fumaric station was even greater. The peaks in this case were broadened probably due to the slow rotation of the macrocycle along the thread and possible folding of the rotaxane.

The significant aspects of the NMR spectra of the *cis* thread **88** and rotaxane **93** are discussed below:

- The effect of having a polar solvent like CD_3OD is much more pronounced in the *cis* system. The succinic amide-ester template has more difficulty competing with this solvent compared to the fumaramide *trans* system. Still, the system shows a higher occupancy with the succinic station with very little occupancy on the maleamide template. Again, discrepancy of the two stations is higher in the rotaxane with the stoppers *t*Bu protected (where it was possible to perform the NMR in $CDCl_3$, a non hydrogen bond competitor). Unfortunately, the NMR peaks very broad in these solvents, and a clear understanding of the functioning of the system in these cases was not possible.
- The protons of the maleamide template are slightly shifted perhaps due to the macrocycle shuttling along the long thread (40Å) as well as the possibility of some folding of the rotaxane on itself. The maleamide template, with only two available hydrogen bonds appears to provide some competing pulling force for the macrocycle.
- Also the alkyl protons are shifted downfield; this is possibly due some back and forth shuttling of the macrocycle along the thread which is detected by the NMR.

3.8.2 Characterization: evaluation of the 'chloride salt' membrane spanning rotaxane.

As previously mentioned we report here only the behavior in solution of the *trans* 'chloride salt' rotaxane **95**. The spectra of thread **89** and rotaxane **95** in CD_3OD (400 MHz, 298K) are shown in Figure 20. The H_f and H_g protons of the fumaramide group are shielded in the rotaxane by approximately 1.23 ppm compared to the

thread, whereas the chemical shifts of the H_p and H_q protons of the succinic amide-ester group are similar in both thread and rotaxane. This indicates that the macrocycle is residing mainly over the fumaramide station. In this system we have a higher occupancy over the fumaramide station compared to the previous system. Moreover, there is an even higher discrimination between the two stations. This remarkable enhancement in selectivity is achieved by modification of the macrocycle which bears an endotopic pyridine in this case, and is responsible of strengthening the hydrogen bonds between the macrocycle and the fumaramide template. As a consequence, the macrocycle appears to have enhanced propensity to reside over the fumaramide station even in strong hydrogen-bond competing solvents like CD_3OD. Again we show that in such system where the stations are 40Å apart there is very good discrimination, and very little shifting was observed by other protons of the rotaxane. Broadening of the peaks in the centre of the spectra may be due to the slow rotation of the macrocycle around the thread encumbered by the 18-crown-6 ether bound to it.

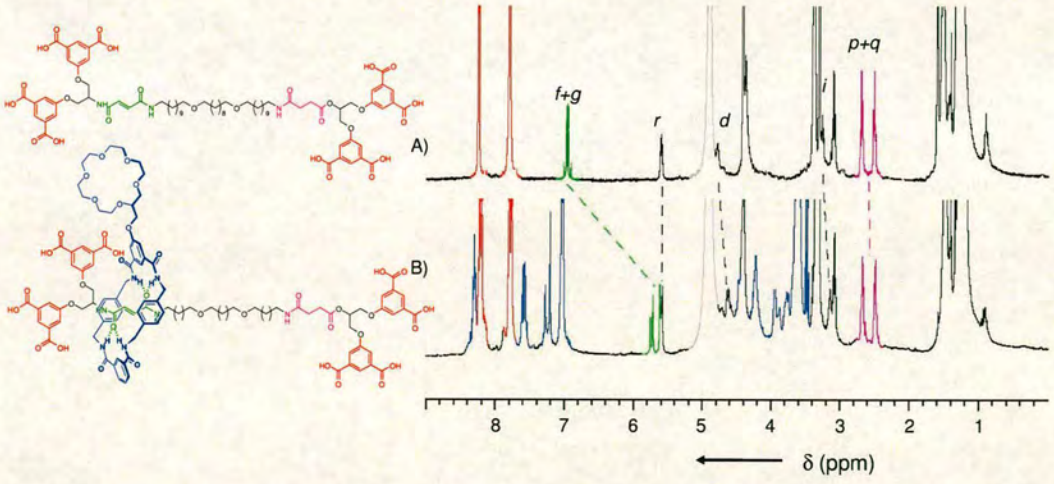


Figure 20. ^1H NMR (400 MHz, 298 K, CD_3OD) spectra of (a) thread **89** and (b) rotaxane **95** (for hydrogen labels refer to scheme 10).

3.9 Conclusion.

Our vision of producing a molecular machine that is capable of delivering ions inside cell is closer to becoming reality. Two different membrane spanning rotaxanes with the potential to transport different ions inside have been designed and synthesized. The shuttling of a macrocycle in the novel system where in the two stations are 40Å (4 nm!) apart has been clearly demonstrated in solution. We anticipate that this system, once imbedded in a membrane, should demonstrate even higher discrimination between the two stations, even in a system where the olefin has a *cis* conformation. The interior of a bilayer should have properties close to that hexane, one of the most non-polar solvents used in synthetic laboratories. Such molecular machines are generating increasing interest, not only as ion transporters, but also in providing synthetic analogues and tools useful for studying the physical properties of bilayers. Smith and coworkers[86] have recently proposed a system based on a similar architecture to that described here which will be used to measure the translocation of phospholipids across vesicle membranes.

The next phase of this project and one equally as challenging, is testing these molecular machines in lipid bilayers. Studies to this end on the rotaxanes described herein are currently being performed by the group of Professor Thomas Fyles at the University of Victoria (Canada).

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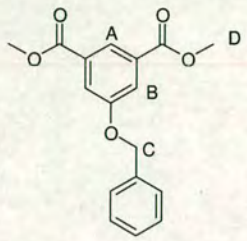
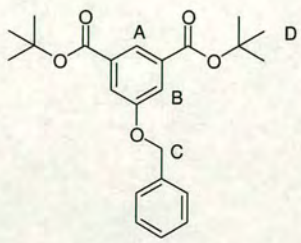
Experimental part

Preparation of 5-Benzyloxy-isophthalic acid dimethyl ester (57).



To a stirred solution **37** (5.0 g, 24 mmol), and K_2CO_3 (20g, 143 mmol) in 2-butanone (150 mL) was added benzyl bromide (11.3 mL, 95 mmol) and the solution was refluxed under nitrogen overnight. K_2CO_3 was filtered, and the solvent was removed by evaporation under reduced pressure. Then, CH_2Cl_2 was added and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Product **57** was isolated by flash chromatography (eluant: CH_2Cl_2, Rf = 0.25); (3.26 g, 76%); mp: 90-92 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 8.29 [t, 1H, J= 1.5 Hz, CH_A], 7.83 [d, 2H, J= 1.5 Hz, CH_B], 7.47-7.31 [m, 5H, H-Ar], 5.13 [s, 2H, CH_C], 3.93 [s, 6H, CH_D]; ^{13}C NMR (100MHz, $CDCl_3$): δ 166.1, 158.8, 136.1, 131.8, 128.7, 127.6, 123.2, 120.2, 70.5, 52.4; FABMS: *m/z* 300 $[M]^+$.

Preparation of 5-Benzyloxy-isophthalic acid di-tert-butyl ester (58).

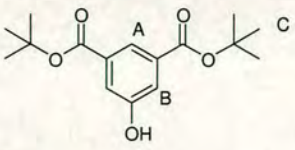


Method a: Compound **57** (1.0 g, 3.3 mmol) and *tert*-butyl acetate (9.0 mL, 67 mmol) were combined in a 100mL Schlenk Flask. The solution was stirred at 40 °C and potassium tert-butoxide (2 mol %, 0.066 mmol, 0.7 mL of a 1M solution in THF) was added via syringe. The reaction was stirred under dynamic vacuum for 15 min to

remove methyl acetate and then backfilled with nitrogen. Additional amounts of tert-butyl acetate (9 mL) and 2 mol % of catalyst were added. The procedure was repeated 4 times. Then, CH_2Cl_2 was added and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Product **58** was isolated by flash chromatography (eluant: CH_2Cl_2/petroleum ether, 7:3, Rf = 0.25). Yield: 55%.

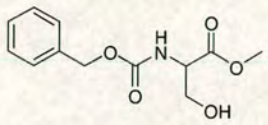
Method b: A stirred solution of 5-hydroxy isophthalic acid (10.0 g, 48 mmol), thionyl chloride (100 mL), and oxalyl chloride (1 mL) was refluxed for 6 hours. Solvents were evaporated and the dry product was combined with an excess of potassium *tert*-butoxide (1M solution in THF) added via syringe. Solvents were removed and then CH_2Cl_2 (500 mL) was added and the solution was washed once with H_2O. The organic solvent was separated from the aqueous phase and evaporated under reduced pressure and then K_2CO_3 (20 g, 143 mmol), 2-butanone (150 mL) and benzyl bromide (11.3 mL, 95 mmol) were added to the crude product. The solution was refluxed under nitrogen overnight. K_2CO_3 was filtered, and the solvent was removed by evaporation under reduced pressure. Then, CH_2Cl_2 was added and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Product **58** was isolated by flash chromatography (eluant: CH_2Cl_2/petroleum ether, 7:3, Rf = 0.25); (12.70 g, 69%); mp: 81-83 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 8.18 [t, 1H, J = 1.5 Hz, CH_A], 7.75 [d, 2H, J = 1.5 Hz, CH_B], 7.47-7.30 [m, 5H, HAr], 5.13 [s, 2H, CH_C], 1.58 [s, 18H, CH_D]; ^{13}C NMR (100MHz, $CDCl_3$): δ165.0, 158.6, 136.4, 133.6, 128.8, 128.3, 127.8, 123.1, 119.6, 81.7, 70.5, 28.2; FABMS: *m/z* 384 $[M]^+$; HRMS: m/z = 384.1984 $[(M)^+]$ (anal. calcd. for $C_{23}H_{28}O_5^+$: *m/z* = 384.1937.

Preparation of 5-Hydroxy-sophthalic acid di-tert-butyl ester (59).



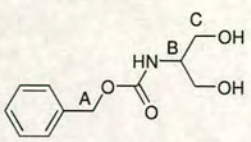
Pd/C (120 mg) was stirred under nitrogen for 5 min then a solution of **4** (1.2 g, 3 mmol) in MeOH (30 mL) was added. Nitrogen was replaced with hydrogen and the solution was stirred for 30 min. Then the catalyst was filtered over a plug of celite and washed with MeOH. The solvent was removed under reduced pressure to afford compound **5** as a white solid (873 mg, 99%); mp: 112-114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 [t, 1H, J =1.5 Hz, CH_A], 7.78 [d, 2H, J = 1.5 Hz, CH_B], 6.30 [brs, 1H, OH], 1.59 [s, 18H, CH_C]; ^{13}C NMR (100MHz, $CDCl_3$): δ 166.3, 157.3, 134.2, 123.3, 121.4, 82.9, 29.0; FABMS: *m/z* 294 $[M]^+$; HRMS: m/z = 294.1463 $[(M)^+]$ (anal. calcd. for $C_{16}H_{22}O_5^+$: *m/z* = 294.1467).

Preparation of 2-N-Carbobenzoxy-D/L-serine methyl ester (60).



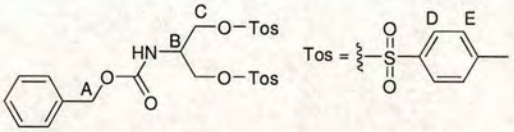
To a 0°C solution of D/L-serine methyl ester hydrochloride (5.0 g, 32 mmol) in $CHCl_3$ (100 mL), Et_3N (9.5 mL 67.5 mmol) was added causing the evolution of gas. Then a solution of Nα-(Benzyloxycarbonyloxy) Succinimide (8.8 g, 35.3 mmol) in $CHCl_3$ (30 mL) was added. The solution was stirred for 4 h at rt. Then $CHCl_3$ was removed under reduced pressure and EtOAc was added (200 mL) and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Product **60** was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 1:1, Rf = 0.25). Yield: 96%. The product showed spectroscopic data according to literature. [1]

Preparation of 2-N-Carbobenzoxy-serinol (61).



To a -78°C solution of **60** (7.0g, 27.4 mmol) in THF (100mL) was added $LiBH_4$ (1.80 g, 82 mmol). Then MeOH (3.3 mL, 82 mmol) was added and the mixture was stirred under an atmosphere of N_2 at room temperature until the reaction was complete by TLC. The reaction was then quenched with 50 mL of H_2O. The organic phase was removed under reduced pressure and EtOAc was added (400 mL) then H_2O was separated. The aqueous layer was backwashed with EtOAc (100 mL) and the organic layers were combined, washed twice with $NaHCO_3$ and once with H_2O and with saturated NaCl dried over anhydrous $MgSO_4$ and evaporated to dryness. Product **61** was isolated by flash chromatography (eluant: CH_2Cl_2/EtOH, 95:5 Rf = 0.25). (4.74 g, 77%); mp: 104-105 °C; ^{1}H NMR (400 MHz, DMSO): δ 7.39-7.28 [m, 5H, H-Ar], 6.87 [d, 1H, J = 8.1 Hz, NH], 5.01 [s, 2H, CH_A], 3.52-3.33 [m, 5H, CH_B and CH_C]; ^{13}C NMR (100MHz, DMSO): δ 157.4, 155.9, 137.2, 128.3, 127.7, 65.2, 60.4, 54.9; FABMS: *m/z* 226 $[M+H]^+$; HRMS: m/z = 226.1074 $[(M+H)^+]$ (anal. calcd. for $C_{11}H_{16}NO_4^+$: *m/z* = 226.1079).

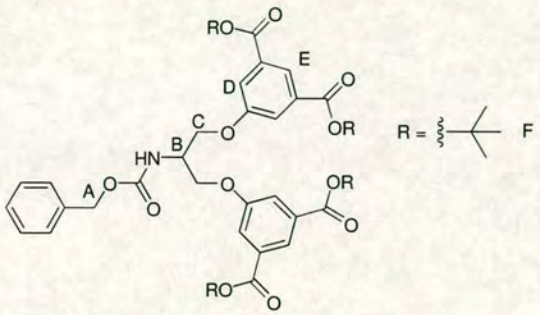
Preparation of 1,3-(Toluene-4-sulfonic acid)-2-benzyloxy carbonylamino-propyl ester (62).



To a 0°C solution of **61** (0.7 g, 3.1 mmol) in $CHCl_3$, pyridine (0.8 mL 10 mmol) was added. Then a solution of p-toluene sulfonyl chloride (1.7 g, 9.3 mmol) in $CHCl_3$ (20mL) was added and the solution was stirred for 12 h at rt. Then $CHCl_3$ was removed under reduced pressure and EtOAc was added (200 mL); the solution was washed twice with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Compound **62** was isolated by flash chromatography (eluant: CH_2Cl_2/EtOH, 99:1 Rf = 0.20). (991 mg, 60%); mp: 100-102 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 [d, 4H, J = 8.3 Hz, CH_E], 7.37-7.24 [m, 9H, CH_E and H-Ar], 5.36 [bd, 1H, J = 8.4 Hz, NH], 5.26 [s, 2H, CH_A], 4.16-3.96 [m, 5H, CH_B and CH_C], 2.41 [s, 6H, CH_3-Ar]; ^{13}C NMR (100MHz, $CDCl_3$): δ 157.8, 145.4, 132.1,

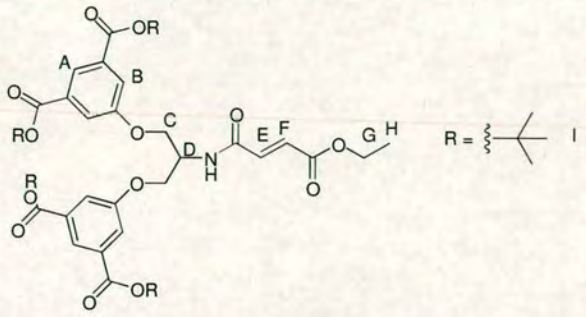
130.1, 128.6, 128.3, 128.1, 128.0, 67.3, 67.0, 48.8, 21.7; FABMS: *m/z* 534 $[M+H]^+$; HRMS: m/z = 534.1243 $[(M+H)^+]$ (anal. calcd. for $C_{25}H_{28}NO_8S_2^+$: *m/z* = 534.1256).

Preparation of [2-(3.5-di-tert-butyl-ester-phenoxy)-1-(3.5-di-tert-butyl-ester-phenoxy)-ethyl]-carbamic acid benzyl ester (63).



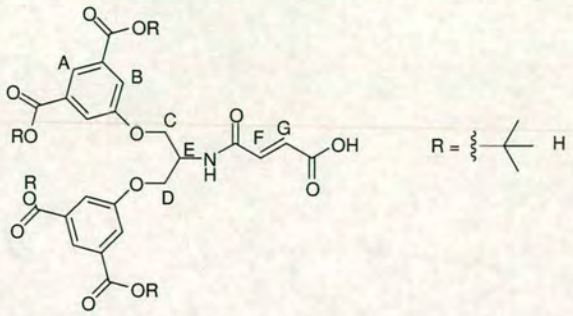
To a stirred solution of **62** (1.9 g, 3.5 mmol), and K_2CO_3 (2.9 g, 21.1 mmol) in 2-butanone (150 mL) was added **59** (3.1 g, 10.6 mmol) and the solution was refluxed under nitrogen for 6h. K_2CO_3 was filtered, and the solvent was removed by evaporation under reduced pressure. Then, CH_2Cl_2 was added and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Compound **62** was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 9:1 Rf = 0.15). (1.33 g, 45%); mp: 88-89 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 8.20 [t, 2H, *J* = 1.5 Hz, CH_E], 7.68 [d, 4H, *J* = 1.5 Hz, CH_D], 7.42-7.30 [m, 5H, H-Ar], 5.47 [d, 1H, *J* = Hz, NH], 5.16 [s, 2H, CH_A], 4.50 [m, 1H, CH_B], 4.35 [dd, 2H, J = 3.7 Hz, J = 9.0 Hz, CH_C], 4.22 [dd, 2H, J = 6.6 Hz, J = 8.8 Hz, CH_C], 1.58 [s, 36H, CH_F]; ^{13}C NMR (100MHz, $CDCl_3$): δ 164.72, 158.0, 155.8, 136.1, 133.5, 128.6, 128.3, 128.2, 123.4, 119.1, 81.1, 67.2, 66.2, 49.6, 28.1; FABMS: *m/z* 776 $[M+H]^+$; HRMS: m/z = 776. 3637 $[(M+H)^+]$ (anal. calcd. for $C_{43}H_{54}NO_{12}^+$: *m/z* = 776.3646).

Preparation of 3-[2-(3,5 di-tert-butyl ester-phenoxy)-1-(3,5 di-tert-butyl ester-phenoxy)-ethylcarbamoyl]-acrylic acid ethyl ester (64).



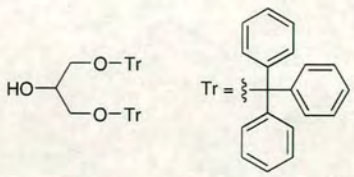
Pd/C (84 mg) was stirred under nitrogen for 5 min then a solution of **63** (0.56 g, 0.7 mmol) in MeOH (30 mL) was added. Nitrogen was replaced with hydrogen and the solution was stirred for 2 h. Then the catalyser was filtered over a plug of celite and washed with MeOH. The solvent was removed under reduced pressure and the solid was dried until the complete removal of MeOH. A solution of the amine and Et_3N (0.24 mL, 1.7 mmol) in anhydrous CH_2Cl_2, was cooled to 0°C, then fumaric acid monoethyl ester chloride (1.7 mmol) in 5mL $CHCl_3$ was added dropwise under nitrogen atmosphere. The solution was allowed to warm up to room temperature and stirred overnight. Then, CH_2Cl_2 was added (100 mL) and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Compound **64** was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 85:15 Rf = 0.20). Yield: 40%; oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 [t, 2H, J = 1.5 Hz, CH_A], 7.68 [d, 4H, J = 1.3 Hz, CH_B], 6.96 [d, 1H, J = 15.4 Hz, CH_F], 6.87 [d, 1H, J = 15.4 Hz, CH_E], 6.33 [d, 1H, J = 8.5 Hz, NH], 4.86-4.79 [m, 1H, CH_D], 4.39-4.20 [m, 6H, CH_C and CH_G], 1.60 [s, 36H, CH_I], 1.32 [t, 3H, J = 7.1 Hz, CH_H]; ^{13}C NMR (100MHz, $CDCl_3$): δ 165.2, 164.7, 163.5, 135.3, 133.6, 132.0, 131.6, 123.5, 119.1, 81.8, 65.9, 61.3, 48.2, 28.1, 14.1; FABMS: *m/z* 768 $[M+H]^+$; HRMS: m/z = 768.3600 $[(M+H)^+]$. (anal. calcd. for $C_{41}H_{54}NO_{13}^+$: *m/z* = 768.3595).

Preparation of 3-[2-(3,5 di-tert-butyl ester-phenoxy)-1-(3,5 di-tert-butyl ester-phenoxy)-ethylcarbamoyl]-acrylic acid (65).



To a stirred solution of **64** (0.90 g, 1.17 mmol), in THF (20 mL) and H_2O (1 mL), was added dropwise a solution of NaOH (60 mg, 1.52 mmol) in H_2O (1.5 mL). After 16 h the solution was reduced in volume and washed several times with Et_2O to obtain compound **65** as a colourless powder. (0.78 g, 96%); mp: 120-122 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 [bt, CH_A], 7.68 [d, 4H, J = 1.3 Hz, CH_B], 7.05 [d, 2H, J = 15.3 Hz, CH_G], 6.90 [d, 2H, J = 15.3 Hz, CH_F], 6.49 [d, 1H, J = 8.5 Hz, NH], 4.83 [m, 1H, CH_E], 4.37 [dd, J = 3.9 Hz, J = 5.4 Hz, CH_C or CH_D], 4.24 [dd, J = 3.9 Hz, J = 5.3 Hz, CH_C or CH_D], 1.60 [s, 36H, CH_H]; ^{13}C NMR (100MHz, $CDCl_3$): δ 168.6, 164.7, 163.2, 157.8, 137.2, 133.6, 130.5, 123.5, 119.1, 81.9, 65.8, 61.3, 48.3, 28.1; FABMS: m/z = 764 $[M+Na]^+$; HRMS: m/z = 764.3257 $[M+Na]^+$ (anal. calcd. for $C_{39}H_{51}NO_{13}Na^+$: m/z = 764.3258).

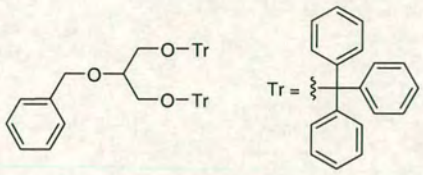
Preparation of 1,3-di-O-tritylglicerol (66).



A mixture containing glycerol (10.0g 108 mmol) and chlorotriphenylmethane (75.7 g, 272 mmol) in pyridine (225mL) was heated at 100°C for 1h. The mixture was cooled to room temperature, poured into ice-water (650 mL) and stirred until a semisolid mass was obtained. The supernatant solution was decanted, and the residue

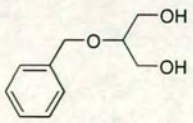
was washed with water (3 x 600 mL) and triturated with warm ethanol (500 mL). The resulting crystalline solid was collected by filtration, washed with ethanol (150 mL), and dried under vacuum to yield an impure sample which was shown by t.l.c. to consist of a major component and traces of minor components. This material was used in subsequent reactions without purification. Yield = 97%. M.p. 170-171 °C. This compound was prepared as described by Depew and coworkers and showed identical spectroscopic data according to literature. [2]

Preparation of 2-O-Benzyl-1,3-di-O-tritylglycerol (67).



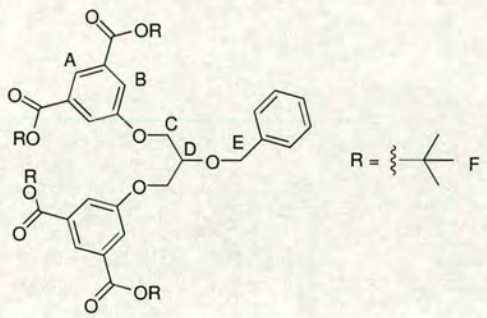
A mixture containing **66** (25.4 g, 44 mmol), Benzyl chloride (32.8 g, 259 mmol) and KOH (25.4 g, 450 mmol) in toluene (200 mL) was heated at reflux temperature for 35 min, cooled to 50°C, diluted with water (200 mL), and the liquid portion extracted with toluene (50 mL). The organic phase was washed with water (40 mL), evaporated, and the oil residue diluted with ethanol (300 mL) and water (30 mL). The mixture was kept at room temperature until a red oil separated (1-2 h). The supernatant solution was decanted and the oil was triturated with warm ethanol (300 mL) to give a red solid. Yield = 81%. This compound was prepared as described by Depew and coworkers and showed identical spectroscopic data according to literature. [2]

Preparation of 2-O-benzyl glycerol (68).



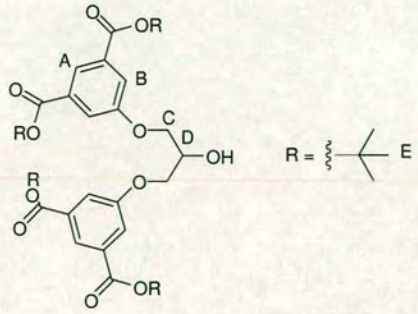
Compound **67** (4.5 g, 6.7 mmol) was suspended in 80% acetic acid (100 mL), heated at 100°C for 30 min, and cooled to room temperature. The triphenylmethanol which precipitated was removed by filtration and washed with 80% acetic acid (25 mL). The combined filtrate and wash solution was evaporated to an oil which, after column chromatography (eluant: CH_2Cl_2/EtOH, 95:5), afforded a white solid. Yield = 51%. M.p. 35-37 °C. This compound was prepared as described by Depew and coworkers and showed identical spectroscopic data according to literature. [2]

Preparation of 2-Benzyloxy-1,3-(3,5 di-tert-butyl ester-phenoxy) propan (69).



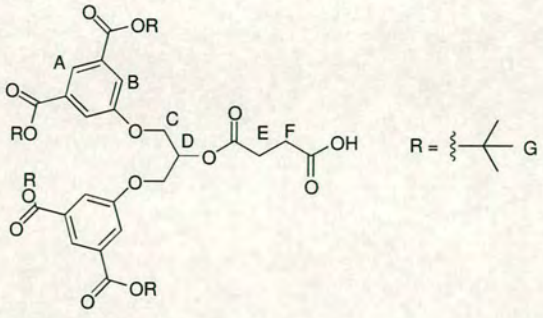
A mixture of **68** (0.57 g, 3.15 mmol) **59** (1.85 g, 6.31 mmol) and triphenylphosphine (2.01 g, 7.9 mmol) in THF (20 mL) is cooled to 0°C. Then diisopropylazadicarboxylate (DIAD) (1.6 mL, 7.9 mmol) is added dropwise and the reaction was stirred overnight at room temperature under nitrogen atmosphere. THF was removed under reduced pressure, CH_2Cl_2 was added (100 mL) and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. Product **69** was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 85:15 Rf = 0.15). (2.04 g, 45%); mp: 60-63 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 [t, 2H, J = 1.5 Hz, CH_A], 7.61 [d, 4H, J = 1.2 Hz, CH_B], 7.39-7.16 [m, 5H, H-Ar], 4.73 [s, 2H, CH_E], 4.28-4.07 [m, 5H, CH_C and CH_D], 1.53 [s, 36H, CH_F]; ^{13}C NMR (100MHz, $CDCl_3$): δ 171.2, 164.9, 158.4, 137.7, 133.5, 128.9, 128.5, 123.2, 119.2, 81.7, 72.6, 60.4, 27.9, 14.2; FABMS: *m/z* 733 $[M+H]^+$; HRMS: m/z = 733.3586 $[(M+H)^+]$ (anal. calcd. for $C_{42}H_{53}O_{11}^+$: *m/z* = 733.3587).

Preparation of 1,3-Bis-(3,5 di-tert-butyl ester-phenoxy)-propan-2-ol (70).



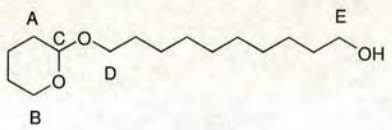
Pd/C (100 mg) was stirred under nitrogen for 5 min then a solution of **69** (0.53 g, 0.72 mmol) in HOAc (30 mL) was added. Nitrogen was replaced with hydrogen and the solution was stirred for 18 h. Then the catalyser was filtered over a plug of celite and washed with MeOH, EtOAc and CH_2Cl_2. The solvent was removed under reduced pressure and the product was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 8:2 Rf = 0.25) to afford compound **70** as a white solid. (348 mg, 75%); mp: 121-123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 [t, 2H, J = 1.5 Hz, CH_A], 7.63 [d, 4H, J = 1.5 Hz, CH_B], 4.48 [quin, 1H, J = 5.1 Hz, CH_D], 4.17 [dd, 4H, J = 2.5 Hz, J = 5.5Hz, CH_C], 2.50 [d, 1H, J = 5.3 Hz, OH], 1.53 [s, 36H, CH_E]; ^{13}C NMR (100MHz, $CDCl_3$): δ 164.8, 157.8, 133.6, 123.4, 119.2, 81.7, 69.0, 68.6, 28.1; FABMS: *m/z* 644 $[M]^+$; HRMS: m/z = 644.3202 $[(M)^+]$ (anal. calcd. for $C_{35}H_{48}O_{11}^+$: *m/z* = 644.3196).

Preparation of Succinic acid mono-[-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester (71).



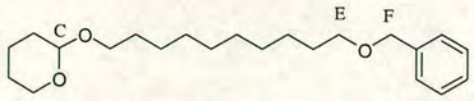
To a stirred solution of **70** (0.23 g, 0.40 mmol) in CH_2Cl_2 (10 mL) was added one drop of Et_3N and a solution of succinic anhydride (44 mg, 0.43 mmol) in CH_2Cl_2 (10 mL) added slowly over 30 mins. After 16 h under nitrogen atmosphere CH_2Cl_2 (30 mL) was added and the solution was washed once with saturated NaCl (5 mL). The aqueous phase was separated and the organic was reduced in volume. Compound **71** was recrystallized from CH_2Cl_2-petroleum ether to obtain a colorless solid. (0.21 g, 90%); mp: 140-142 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 [t, 2H, *J* = 1.5 Hz, CH_A], 7.67 [d, 4H, *J* = 1.5 Hz, CH_B], 5.55 [quin, 1H, *J* = 5.1 Hz, CH_D], 4.34 [d, 4H, *J* = 5.3 Hz, CH_C], 2.70 [m, 4H, CH_E and CH_F], 1.60 [s, 36H, CH_G]; ^{13}C NMR (100MHz, $CDCl_3$): δ 171.5, 164.8, 158.2, 157.8, 133.6, 123.5, 119.4, 81.8, 70.4, 66.4, 29.0, 28.5, 28.1; FABMS: *m/z* 767 $[M+Na]^+$; HRMS: m/z = 767.3262 $[(M+Na)^+]$ (anal. calcd. for $C_{39}H_{52}NaO_{14}^+$: *m/z* = 767.3254).

Preparation of 10-(Tetrahydro-pyran-2-yloxy)-decan-1-ol (72).



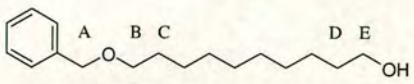
A mixture of decane-1,10-diol (10 g, 57 mmol), aqueous 5M $NaHSO_4$ (58 mL), DMSO (12 mL) and 3:97 (vol/vol) DHP-hexane was stirred for 5 h at 40°C. The solvents were removed under reduced pressure and then CH_2Cl_2 was added and the solution was washed with 1N HCl, dried over anhydrous $MgSO_4$, and evaporated to dryness. The product was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 7:3 Rf= 0.20). Mp = 45-46 °C. Yield 70%, oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.47 [dd, 1H, *J* = 2.5 Hz, *J* = 4.0 Hz CH_C], 3.75 [ddd, 1H, *J* = 3.2, 7.5, 11.0 Hz, CH_A], 3.61 [dt, 1H, *J* = 6.8 Hz, *J* = 9.3 Hz], 3.48 [t, 2H, *J* = 6.5Hz CH_E], 3.40 [ddd, 1H, *J* = 3.2, 7.5, 10.5 Hz], 3.27 [dt, 1H, *J* = 6.8 Hz, *J* = 9.6 Hz], 2.76 [s, 1H, OH], 1.76-1.55 [m, 2H, THP-CH_2], 1.52-1.36 [m, 8H, CH_2 alkyl and THP-CH_2], 1.28-1.14 [m, 12H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 97.9, 66.8, 61.7, 61.4, 31.9, 29.9, 28.8, 28.7, 28.6, 25.4, 24.9, 24.6, 18.7; FABMS: *m/z* 259 $[M+H]^+$; HRMS: m/z = 259.2271 $[(M+H)^+]$ (anal. calcd. for $C_{15}H_{31}O_3^+$: *m/z* = 259.2273).

Preparation of 2-(10-Benzyloxy-decyloxy)-tetrahydro-pyran (73).



To a mixture of **72** (8.00 g, 31 mmol) in DMF were added 60% sodium hydride (1.60 g, 40 mmol), benzyl bromide (7.4 mL, 62 mmol) and tetrabutyl ammonium iodide (0.10 g, 0.3 mmol) for 4 h. The reaction was quenched with H_2O (30 mL) and the solvent was removed under reduced pressure. Then CH_2Cl_2 was added and the organic layer was washed twice with $NaHCO_3$ and once with H_2O and with saturated NaCl dried over anhydrous $MgSO_4$, and evaporated to dryness. Compound **20** was isolated by flash chromatography (eluant: petroleum ether/Et_2O, 95:5, Rf = 0.20). Yield: 76%; Oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.25 [m, 5H, H-Ar], 4.59 [t, 1H, J = 3.5 Hz, CH_C], 4.50 [s, 2H, CH_F], 3.88 [ddd, 1H, J = 2.7, 7.5, 10.7 CH], 3.75 [dt, 1H, J = 6.7 Hz, J= 9.3 CH], 3.54-3.43 [m, 1H, CH], 3.47 [t, 2H, J = 6.6 Hz, CH], 3.39 [dt, 1H, J = 6.7 Hz, J = 9.3 Hz, CH], 1.91-1.70 [m, 2H, THP-CH_2], 1.67-1.47 [m, 8H, CH_2 alkyl and THP-CH_2], 1.38-1.32 [m, 12H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 157.3, 138.7, 128.3, 127.8, 127.4, 98.8, 72.8, 70.5, 68.8, 67.7, 62.3, 30.8, 30.5, 29.8, 29.5, 26.3, 26.2, 25.5, 19.7; FABMS: *m/z* 347 $[M+H]^+$; HRMS: m/z = 347.2589 $[(M+H)^+]$ (anal. calcd. for $C_{22}H_{35}O_3^+$: *m/z* = 347.2586).

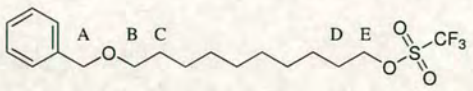
Preparation of 10-Benzyloxy-decan-1-ol (74).



A solution of **73** (7.1 g, 20.4 mmol) and pyridinium p-toluenesulfonate (PPTS) (0.5 g, 2 mmol) in EtOH (200 mL) was stirred at 60 °C for 5 h. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography (eluant: petroleum ether/EtOAc, 7:3, Rf = 0.25). to afford a pure alcohol. Yield = 81%. Oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.26 [m, 5H, H-Ar], 4.50 [s, 2H, CH_A], 3.63 [t, 2H, J = 6.8 Hz CH_E], 3.46 [t, 2H, J = 6.6 Hz, CH_B], 1.64-

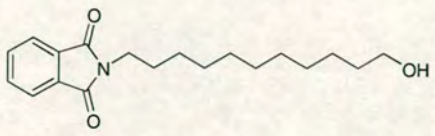
1.52 [m, 4H, CH_C and CH_D], 1.35-1.26 [m, 12H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 138.7, 128.3, 127.6, 127.4, 72.9, 70.5, 63.1, 32.8, 29.8, 29.5, 29.4, 26.2, 25.7; FABMS: *m/z* 265 $[M+H]^+$; HRMS: m/z = 265.2171 $[M+H]^+$ (anal. calcd. for $C_{17}H_{29}O_2^+$: *m/z* = 265.2167).

Preparation of Trifluoro-methanesulfonic acid 10-benzyloxy-decyl ester (75).



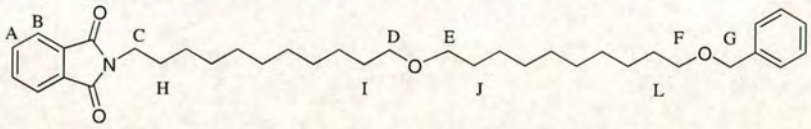
To an ice-cooled flask containing CH_2Cl_2 (100 mL) under nitrogen atmosphere was added Tf_2O (2.4 mL, 14.0 mmol), followed by anhydrous pyridine (1.1 mL, 14.0 mmol). Fuming was observed and a white precipitate formed. The cooling bath was removed and a solution of **74** (2.7 g, 10 mmol) in CH_2Cl_2 (20 mL) was added dropwise over a period of 10 min with stirring. The solution was stirred for 2h at room temperature and then water (20 mL) was added to quench the reaction. CH_2Cl_2 (200 mL) was added, and the solution was washed twice with water (2 x 100 mL). The aqueous layer were backwashed with CH_2Cl_2 (20 mL) and the organic layer were combined, washed with brine (100 mL), dried over $MgSO_4$, and evaporated under reduced pressure to yield a brown oil. The oil was dissolved in a mixtureof CH_2Cl_2/ petroleum ether 1:1, and loaded onto a 1 in. bed of silica. The product was eluted with hexane and diethyl ether, taking care not to coelute the more polar, colored byproducrs. The solvents were removed under reduced pressure to furnish compound **75** as colourless oil. Yield = 85%. Oil. Due to instability of the product only the ^1H NMR is shown.^1H NMR (400 MHz, $CDCl_3$): δ 7.42-7.26 [m, 5H, H-Ar], 4.56 [t, 2H, *J* = 6.3 Hz, CH_E], 4.54 [s, 2H, CH_A], 3.50 [t, 2H, *J* = 6.6 Hz, CH_B], 1.85 [quin, 2H, *J* = 6.8 Hz, CH_D], 1.65 [quin, 2H, *J* = 6.6Hz, CH_C], 1.49-1.28 [m, 12H, CH_2 alkyl].

Preparation of 11-hydroxyundecyl-1-phthalimide (76).



11-bromo undecanol (10.4 g, 40 mmol) and potassium phthalimide (9.3 g, 50 mmol) were suspended in 100 mL of DMF and the mixture was stirred for 18 h at 80°C under nitrogen atmosphere. Then the solid precipitated was filtrated and solvent was removed under reduced pressure. CH_2Cl_2 was added (300 mL) and the solution was washed with 1M HCl (50 mL), H_2O, 5% $NaHCO_3$ (50 mL), H_2O (100 mL), dried over anhydrous $MgSO_4$, and evaporated to dryness to give a white solid. Yield: 95% Mp = 85-86 °C. FABMS: *m/z* 318 $[M+1]^+$. This compound was prepared as described by Ihara and coworkers and showed identical spectroscopic data according to literature. [3]

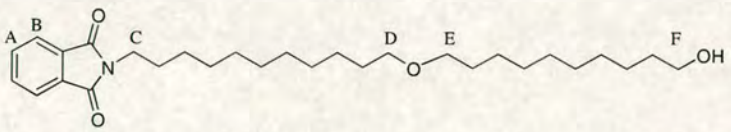
Preparation of 2-[11-(10-benzyloxy-decyloxy)-undecyl]-isoindole-1,3-dione (77).



To a mixture of **75** (1.10 g, 3.5 mmol), **76** (2.50 g, 6.3 mmol) and 1,8-bis(dimethylamino)naphthalene (Proton Sponge, 1.35 g, 6.3 mmol) under nitrogen atmosphere was added CH_2Cl_2 (30 mL). The yellow solution was refluxed under nitrogen for 78 h. During this time, the reaction had turned dark orange/brown, and a precipitate had formed. Then, CH_2Cl_2 was added and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. The product was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 8:2 Rf = 0.20). Yield: 88%. Oil. ^1H NMR (400 MHz, $CDCl_3$): δ 7.83 [dd, 2H, J = 5.8 Hz, J = 2.7 Hz, CH_B], 7.69 [dd, 2H, J = 3.0 Hz, J = 5.5 Hz, CH_A], 7.39-7.25 [m, 5H, H-Ar], 4.50 [s, 2H, CH_G], 3.67 [t, 2H, J = 7.0 Hz, CH_C], 3.47 [t,

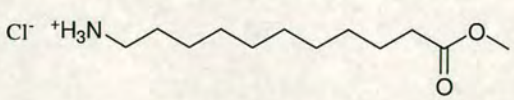
2H, J = 6.8 Hz, CH_F], 3.39 [t, 4H, J = 6.8 Hz, CH_D and CH_E], 1.71-1.51 [m, 8H, $CH_{H,I,J,L}$], 1.33-1.26 [m, 26H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 168.2, 138.5, 133.6, 132.0, 128.1, 127.4, 127.2, 122.9, 72.6, 70.7, 70.7, 70.3, 37.8, 29.6, 29.5, 29.3, 29.3, 29.2, 29.0, 28.4, 26.6, 26.0; FABMS: *m/z* 564 $[M+H]^+$; HRMS: m/z = 564.4055 $[M+H]^+$ (anal. calcd. for $C_{36}H_{54}NO_4^+$: *m/z* = 564.4052).

Preparation of 2-[11-(10-hydroxy-decyloxy)-undecyl]-isoindole-1,3-dione (78).



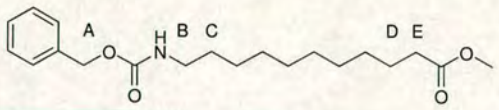
Pd/C (100 mg) was stirred under nitrogen for 5 min then a solution of **77** (2.80 g, 5.0 mmol) in HOAc (50 mL) was added. Nitrogen was replaced with hydrogen and the solution was stirred for 18 h. Then the catalyser was filtered over a plug of celite and washed with MeOH, EtOAc and CH_2Cl_2. The solvent was removed under reduced pressure, CH_2Cl_2 was added and the solution was washed with 1N HCl, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness and the product was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 1:1 Rf = 0.25) to afford a white solid. Yield = 75 %. Oil. ^1H NMR (400 MHz, $CDCl_3$): δ 7.83 [dd, 2H, J = 3.0 Hz, J = 5.5 Hz, CH_B], 7.70 [dd, 2H, J = 3.2 Hz, J = 5.3 Hz, CH_A], 3.68-3.61 [m, 4H, CH_C and CH_F], 3.37 [t, 4H, J = 6.8 Hz CH_D and CH_E], 1.61-1.48 [m, 8H, CH_2 alkyl], 1.30-1.14 [m, 26H, CH_2 alky]; ^{13}C NMR (100MHz, $CDCl_3$): δ 168.5, 133.8, 132.2, 123.1, 70.9, 70.9, 63.1, 53.4, 38.1, 32.8, 29.8, 29.5, 29.4, 29.2, 28.6, 26.9, 26.2, 25.7; FABMS: *m/z* 474 $[M+H]^+$; HRMS: m/z = 474.3580 $[M+H]^+$ (anal. calcd. for $C_{29}H_{48}NO_4^+$: *m/z* = 474.3583).

Preparation of 11-Amino-undecanoic acid methyl ester hydrochloride (79).



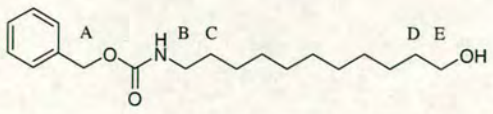
To a mixture of 11 amino undecanoic acid (10 g, 50 mmol) and methanol 120, thionyl chloride (5.8 mL, 80 mmol) was added dropwise over a period of 10 min with stirring. The solution was refluxed for 2h temperature and then for other 2 h at room temperature. The solvent is evaporated under reduced pressure and the white solid is filtered and washed with diethyl ether. Yield = 99%. This compound was prepared as described by De Clercq and coworkers and showed identical spectroscopic data according to literature. [4]

Preparation of 11-Benzyloxycarbonylamino-undecanoic acid methyl ester (80).



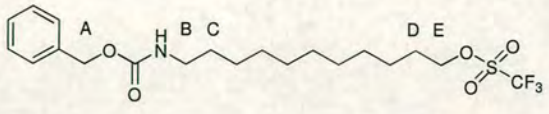
To a 0°C solution of **80** (5.0 g, 23 mmol) in $CHCl_3$ (80mL), TEA (6.8 mL 48.4 mmol) was added causing the evolution of gas. Then a solution of Nα-(Benzyloxycarbonyloxy) Succinimide (6.34 g, 25.4 mmol) in $CHCl_3$ (30mL) was added. The solution was stirred for 4 h at rt. Then $CHCl_3$ was removed under reduced pressure and CH_2Cl_2 was added (200 mL) and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. The product was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 2:8 Rf = 0.25). (7.20 g, 90%); mp: 92-95 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.23 [m, 5H, H-Ar], 5.09 [s, 2H, CH_A], 4.72 [brt, 1H, NH], 3.66 [s, 3H, O-Me CH_3], 3.17 [t, 2H, J = 6.8Hz, CH_B], 2.29 [t, 2H, J = 7.5 Hz, CH_E] 1.61 [quin, 2H, J = 7.1 Hz, CH_C], 1.48 [quin, 2H, J = 6.5 Hz, CH_D], 1.33-1.21 [m, 12H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 174.3, 156.4, 136.7, 128.5, 128.5, 128.1, 66.6, 51.4, 41.1, 34.1, 29.9, 29.3, 29.2, 29.1, 26.7, 24.9; FABMS: *m/z* 350 $[M+H]^+$; HRMS: m/z = 350.2328 $[M+H]^+$ (anal. calcd. for $C_{20}H_{32}NO_4^+$: *m/z* = 350.2331).

Preparation of (11-Hydroxy-undecyl)-carbamic acid benzyl ester (81).



To a -78°C solution of **80** (6.8 g, 19.5 mmol) in THF (100mL) was added $LiBH_4$ (1.27g, 58.4 mmol). Then MeOH (2.4 mL, 58.4 mmol) was added and the mixture was stirred under an atmosphere of N_2 at room temperature until the reaction was complete by TLC. The reaction was then quenched with 50 mL of H_2O. The organic phase was removed under reduced pressure and EtOAc was added (400 mL) then H_2O was separated. The aqueous layer was backwashed with AcOEt (100mL) and the organic layer were combined, washed twice with $NaHCO_3$ and once with H_2O and with saturated NaCl dried over anhydrous $MgSO_4$, and evaporated to dryness. Product **81** was isolated by flash chromatography (eluant: petroleum ether/EtOAc, 6:4, Rf = 0.25). Mp = 121-122 °C. Yield: 77%. Oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.28 [m, 5H, H-Ar], 5.09 [s, 2H, CH_A], 4.75 [brt, 1H, NH], 3.63 [t, 2H, J = 6.8 Hz, CH_E], 3.17 [t, 2H, J = 6.8 Hz, CH_B], 1.55 [quin, 2H, J = 6.8 Hz, CH_C], 1.48 [quin, 2H, J = 6.3 Hz, CH_D], 1.37-1.21 [m, 14H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 157.8, 136.7, 128.5, 128.5, 128.1, 66.6, 63.0, 41.1, 32.8, 30.0, 29.5, 29.4, 29.2, 26.7, 25.7; FABMS: *m/z* 322 $[M+H]^+$; HRMS: m/z = 322.2383 $[M+H]^+$ (anal. calcd. for $C_{19}H_{32}NO_3^+$: *m/z* = 322.2382).

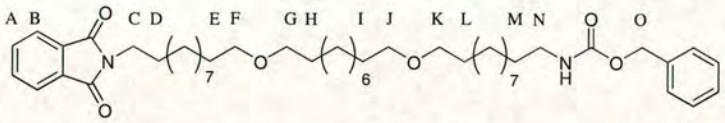
Preparation of Trifluoro methanesulfonic acid 11-benzyloxycarbonyalmino-undecyl ester (82).



To a –78°C cooled flask containing CH_2Cl_2 (30 mL) under nitrogen atmosphere was added Tf_2O (1.9 mL, 11.3 mmol), followed by anhydrous pyridine (0.9 mL, 11.3 mmol). Fuming was observed and a white precipitate formed. Then a solution of **81** (2.6 g, 8.1 mmol) in CH_2Cl_2 (20 mL) was added dropwise over a period of 10 min

with stirring. The solution was stirred for 7 h at –78°C and then water (20 mL) was added to quench the reaction. CH_2Cl_2 (200 mL) was added, and the solution was washed twice with water (2 x 100 mL). The aqueous layer were backwashed with CH_2Cl_2 (20 mL) and the organic layer were combined, washed with brine (100 mL), dried over $MgSO_4$, and evaporated under reduced pressure to yield a brown oil. The oil was dissolved in a mixture of CH_2Cl_2/ petroleum ether 1:1, and loaded onto a 1 in. bed of silica. The product was eluted with hexane and diethyl ether, taking care not to coelute the more polar, coloured byproducts. The solvents were removed under reduced pressure to give compound **82** as colorless oil. Yield = 70%. Oil. ^{1}H NMR (400 MHz, $CDCl_3$): δδ 7.38-7.29 [m, 5H, H-Ar], 5.10 [s, 2H, CH_A], 4.70 [brt, 1H, NH], 4.53 [t, 2H, J = 6.5 Hz, CH_E], 3.18 [t, 2H, J = 7.5 Hz, CH_B], 1.82 [quin, 2H, J = 7.0 Hz, CH_C], 1.55-1.35 [m, 4H, CH_D and CH_2 alkyl], 1.35-1.17 [m, 12H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 156.5, 136.6, 128.8, 128.1, 123.4, 77.7, 63.2, 41.2, 31.6, 31.3, 31.2, 29.9, 29.0, 28.8, 28.7, 26.1, 25.2.

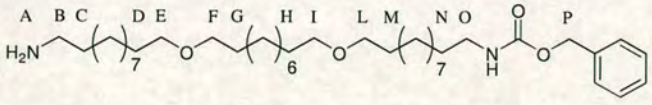
Preparation of (11-{10-[11-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-undecyloxy]-decyloxy}-undecyl)-carbamic acid benzyl ester (83).



To a mixture of **78** (1.50 g, 3.10 mmol), **82** (2.6 g, 5.66 mmol) and 1,8-bis(dimethylamino)naphthalene (Proton Sponge, 1.21 g, 5.66 mmol) under nitrogen atmosphere was added CH_2Cl_2 (30 mL). The yellow solution was refluxed under nitrogen for 78 h. During this time, the reaction had turned dark orange/brown, and a precipitate had formed. Then, CH_2Cl_2 was added and the solution was washed with 1M HCl, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous $MgSO_4$,and evaporated to dryness. Compound **83** was isolated by flash chromatography (eluant: petroleum hexane/diethyl ether, 8:2, Rf = 0.20). (1.45 g, 60%); mp: 1-1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 [dd, 2H, J = 3.0 Hz, J = 5.3 Hz, CH_B], 7.70 [dd, 2H, J = 3.2 Hz, J = 5.6 Hz, CH_A], 7.36-7.30 [m, 5H, H-Ar], 5.09 [s, 2H, CH_O], 4.74 [brt, 1H, NH], 3.66

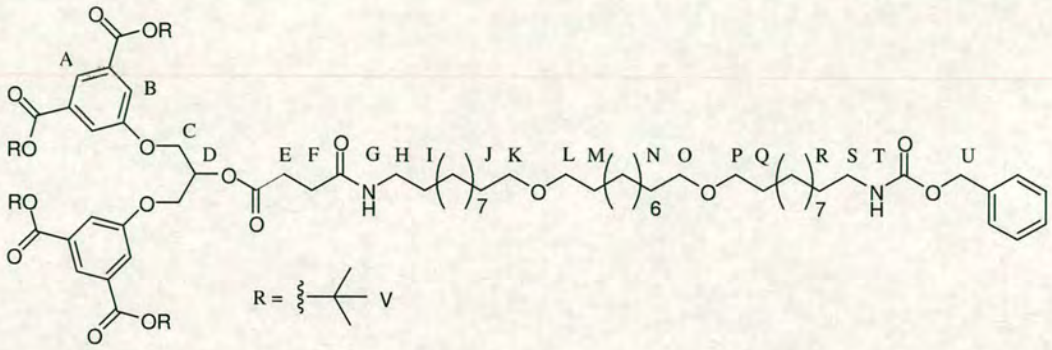
[t, 2H, J = 7.3 Hz, CH_C], 3.37 [t, 8H, J = 6.5 Hz, CH_{FGJK}], 3.18 [t, 2H, J = 6.5 CH_N], 1.71-1.42 [m, 12H, CH_{DEHILM}], 1.37-1.28 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 168.4, 157.8, 156.4, 136.7, 133.80, 132.2, 128.5, 128.10, 123.1, 71.0, 71.0, 71.0, 71.0, 66.5, 41.1, 38.1, 30.0, 29.8, 29.5, 29.3, 29.2, 28.6, 26.9, 26.7, 26.2; FABMS: *m/z* 777 $[M+H]^+$; HRMS: m/z = 777.5788 $[M+H]^+$ (anal. calcd. for $C_{48}H_{77}N_2O_6^+$: *m/z* = 777.5781).

Preparation of {11-[10-(11-Amino-undecyloxy)-decyloxy]-undecyl}-carbamic acid benzyl ester (84).



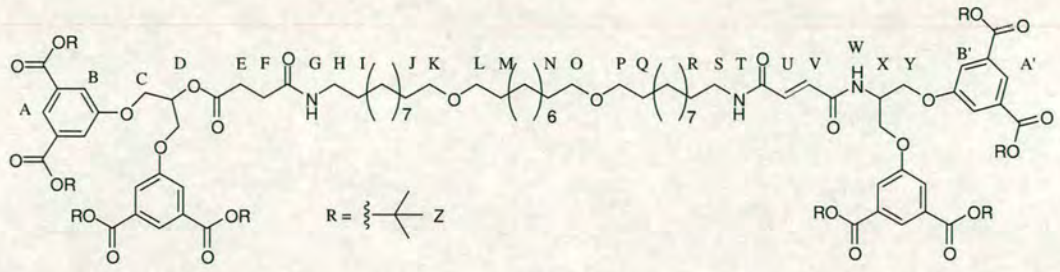
A solution of **83** (1.0 g, 1.28 mmol) and hydrazine (0.25 mL, 5.15 mmol) in absolute ethanol was refluxed for 3 h. The voluminous white precipitate was separated by filtration (2x), and then the filtrate was concentrated in vacuo to an off-white solid. The white solid was triturated twice with $CHCl_3$ (200 mL) and filtered. The filtrate was then dried over anhydrous $MgSO_4$. After filtration of the inorganic salt the filtrate was concentrated in vacuo to yield compound **84** a glassy white solid. (0.79 g, 96%); mp: 99-103 °C; ^1H NMR (400MHz, $CDCl_3$): δ 7.35-7.04 [m, 5H, H-Ar], 5.00 [s, 2H, CH_P], 4.72 [brt, 1H, NH], 3.29 [t, 8H, J = 6.7 Hz, CH_{EFIL}], 3.07 [td, 2H, J = 6.6Hz, J = 6.3 Hz, CH_O], 2.50 [t, 2H, J = 7.0, CH_B], 1.52-1.29 [m, 12H, CH_{CDGHMN}], 1.29-1.06 [m, 40H, CH_2 alkyl]; ^{13}C NMH (100MHz, $CDCl_3$): 156.4, 136.7, 128.4, 128.1, 128.0, 71.2, 66.5, 42.1, 41.1, 33.5, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 26.8, 26.7, 26.1; FABMS; 647 *m/z* $[M+H]^+$; HRMS : *m/z* = 647.5711 $[M+H]^+$ (anal. calcd. for $C_{40}H_{75}N_2O_4^+$: *m/z* = 647.5726).

Preparation of N-{11-[10-(11-Amino-undecyloxy)-decyloxy]-undecyl}-succinamic acid -[-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester (85).



To a stirred solution of **84** (0.84 g, 1.3 mmol) and **71** (1.09 g, 1.43 mmol[COOH]) and HOBt (340 mg, 2.5 mmol) and Et_3N (0.57 mL, 2.6 mmol) in anhydrous CH_2Cl_2 (150 mL) cooled in a ice bath, was added EDCI·HCl (0.48 mg, 2.50 mmol) and the reaction was allowed to stir for 48 h at rt. The solution was washed twice with 1N HCl, 5% $NaHCO_3$, H_2O, and dried over anhydrous $MgSO_4$ and evaporated to dryness. Compound **85** was isolated by flash chromatography (eluant : CH_2Cl_2/MeOH, 99:1, Rf = 0.20). (1.37 g, 77%); oil; ^1H NMR (400MHz, $CDCl_3$): δ 8.16 [s, 2H, CH_A], 7.65 [d, 4H, J = 0.9 Hz, CH_B], 7.36-7.25 [m, 5H, H-Ar], 5.63 [bt, 1H, NH_G], 5.50 [quin, 1H, J = 4.9 Hz, CH_D], 5.06 [s, 2H, CH_U], 4.72 [bt, 1H, NH_T], 4.31 [d, 4H, J = 4.9 Hz, CH_C], 3.35 [t, 8H, J = 6.7 Hz, CH_{KLOP}], 3.16 [m, 4H, CH_H], 2.72 [t, 2H, J = 6.9 Hz, CH_E], 2.45 [t, 2H, J = 6.8 Hz, CH_F], 1.57 [s, 36H, CH_V], 1.55-1.40 [m, 12H, CH_{IJMNQR}], 1.36-1.10 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100MHz, $CDCl_3$): δ 172.3, 170.8, 164.7, 158.1, 136.6, 133.5, 128.4, 128.1, 123.3, 119.2, 81.7, 70.9, 66.2, 41.1, 39.7, 31.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 28.1, 26.7, 26.1; FABMS: *m/z* $[M+H]^+$ 1373; HRMS: *m/z* = 1373.8995 $[M+H]^+$ (anal. calcd. for $C_{79}H_{125}N_2O_{17}^+$: *m/z* = 1373.8978).

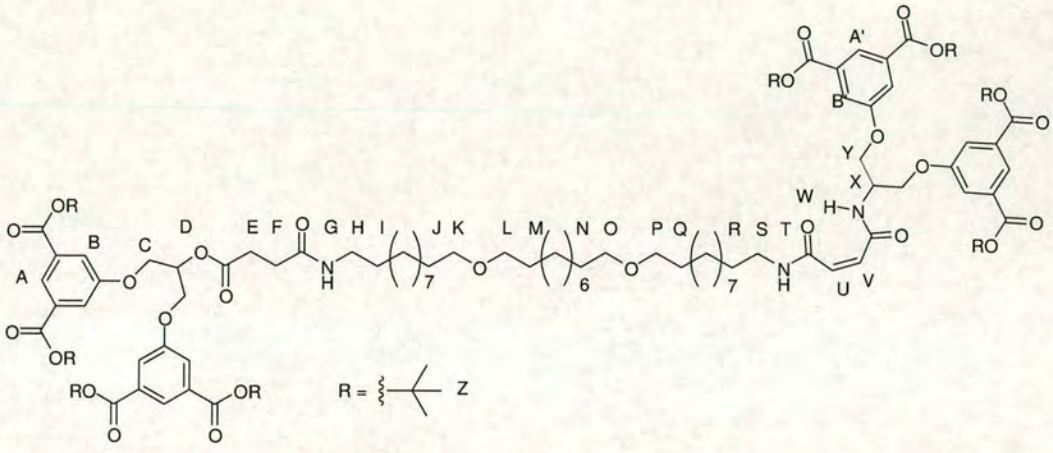
Preparation of {E}-N-[11-(10-{11-[3-(-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester (86).



A solution of **85** (695 mg, 0.51 mmol) in MeOH/THF (20 mL, 9:1) was hydrogenated using 20 % Pd/C (100 mg) for 4 h at atmospheric pressure and the disappearance of starting material was monitored by TLC. The reaction mixture was filtered through Celite, washed with MeOH (15 mL) and concentrated under reduced pressure to furnish a colorless solid on cooling, which was used without any further purification. Carboxylic acid **65** (450 mg, 0.76 mmol) was dissolved in CH_2Cl_2 (20 mL) and the solution was cooled to 0 °C. Bop (505 mg, 1.15 mmol) and Et_3N (0.23 mL, 1.65 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 30 min. A solution of the above amine in dichloromethane (10mL) was added to the activated acid. The reaction mixture was stirred for 48 h, diluted with CH_2Cl_2 (10mL) and washed once with 1M HCL and 5% $NaHCO_3$. The crude material was purified by flash chromatography (eluant: CH_2Cl_2/CH_3CN, 9:1) to furnish compound **86** as colorless solid. Mp = °C. Yield: 60%. Oil. ^1H NMR (400MHz, $CHCl_3$) δ = 8.20-8.15 [m, 4H, $CH_{AA'}$], 7.70-7.60 [2d, 8H, $CH_{BB'}$], 6.98 [d, 1H, J = 14.7 Hz, CH_U or CH_V], 6.93 [d, 1H, J = 14.8 Hz, CH_U or CH_V], 6.65 [d, 1H, J = 8.4 Hz, NH_W], 6.09 [t, 1H, J = 5.8 Hz, NH_T], 5.71 [t, 1H, J = 5.4 Hz, NH_G], 5.53 [quin, 1H, J = 4.8 Hz, CH_D], 4.80 [m, 1H, CH_X], 4.40-4.27 [m, 6H, CH_C, CH_C and CH_Y], 4.22 [dd, 2H, J = 3.1 Hz, J = 6.0 Hz, CH_Y], 3.37 [t, 8H, J = 6.7 Hz, CH_{KLOP}], 3.32 [q, 2H, J = 6.7 Hz, CH_S], 3.20 [q, 2H, J = 6.7 Hz, CH_H], 2.74 [t, 2H, J = 6.9 Hz, CH_E], 2.48 [t, 2H, J = 6.7 Hz, CH_F], 1.59 [s, 36H, CH_Z], 1.56 [s, 36H, CH_Z], 1.57-1.48 [m, 12H, CH_{IJMNQR}], 1.36-1.17 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100MHz,

$CDCl_3$): δ = 172.3, 170.8, 164.7, 164.6, 164.1, 163.7, 158.1, 157.8, 134.0, 133.5, 132.2, 123.4, 119.2, 119.0, 81.7, 70.9, 70.2, 66.2, 65.8, 53.4, 48.2, 39.9, 39.6, 31.0, 29.8, 29.7, 29.6, 29.52, 29.48, 29.4, 29.3, 29.25, 29.2, 28.1, 26.9, 26.8, 26.1; FABMS: *m/z* = 1964 $[M]^+$; HRMS: *m/z* = 1964.1955 $[M]^+$ (anal. calcd. for $C_{110}H_{169}N_3O_{27}^+$: *m/z* = 1964.1943).

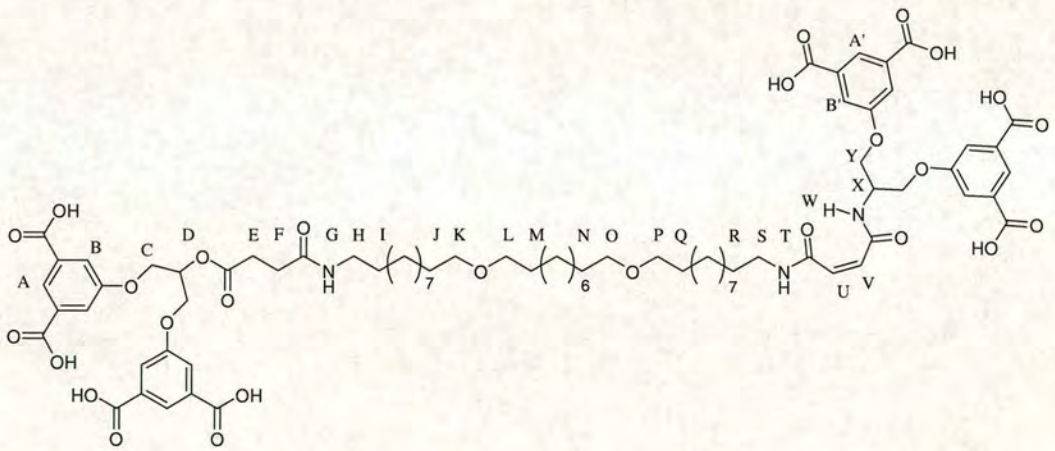
Preparation of {Z}-N-[11-(10-{11-[3-(-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester (87).



The fumaramide derivative **86** (0.05 mmol) was dissolved in CH_2Cl_2 (30 mL) in a quartz vessel. The solution was directly irradiated at 254 nm using a multilamp photo-reactor. The progress of the photoisomerization was monitored by TLC [CH_2Cl_2/MeOH (95/5)] or ^1H NMR. Different photostationary states were reached in a range of times not exceeding 20 min, after which the reaction mixture was concentrated under reduce pressure to afford the crude product. The crude material was purified by flash chromatography (eluant: CH_2Cl_2/MeOH, 95:5) to furnish compound **87** as colorless oil. Yield: 30%. Oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.39 [d, 1H, *J* = 7.8 Hz, NH_W], 8.18 [t, 2H, CH_A or $CH_{A'}$], 8.17 [t, 2H, CH_A or $CH_{A'}$], 7.70 [d, 4H, *J* = 1.1 Hz, CH_B or $CH_{B'}$], 7.66 [d, 4H, *J* = 1.2 Hz, CH_B or $CH_{B'}$], 7.45 [bt, 1H, NH_T], 6.16 [d, 1H, *J* = 13.5 Hz, CH_U or CH_V], 6.11 [d, 1H, *J* = 13.5 Hz,

CH_U or CH_V], 5.70 [t, 1H, J = 5.5 Hz, NH_G], 5.53 [quin, 1H, J = 4.8, CH_D], 4.74 [m, 1H, CH_X], 4.40-4.29 [m, 6H, CH_C, CH_C and CH_Y], 4.28-4.20 [m, 2H, CH_Y], 3.37 [t, 8H, J = 6.7 Hz, CH_{KLOP}], 3.29 [q, 2H, J = 6.5 Hz, CH_S], 3.20 [q, 2H, J = 6.7 Hz, CH_H], 2.74 [t, 2H, J = 6.9 Hz, CH_E], 2.48 [t, 2H, J = 6.8 Hz, CH_F], 1.59-1.57 [s, 72H, CH_Z], 1.55-1.40 [m, 12H. CH_{IJMNQR}], 1.35-1.17 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.3, 170.8, 164.7, 158.1, 157.7, 133.5, 133.4, 123.4, 123.2, 119.2, 119.0, 81.7, 70.9, 70.2, 66.2, 65.9, 39.7, 30.9, 29.8, 29.7, 29.6, 29.5 29.4, 29.3, 29.2, 29.0, 28.1, 26.9, 26.8; FABMS: m/z = 1964 $[M]^+$; HRMS: m/z = 1964.1898; $[(M)^+]$ (anal. calcd. for $C_{110}H_{169}N_3O_{27}^+$: m/z = 1964.1943).

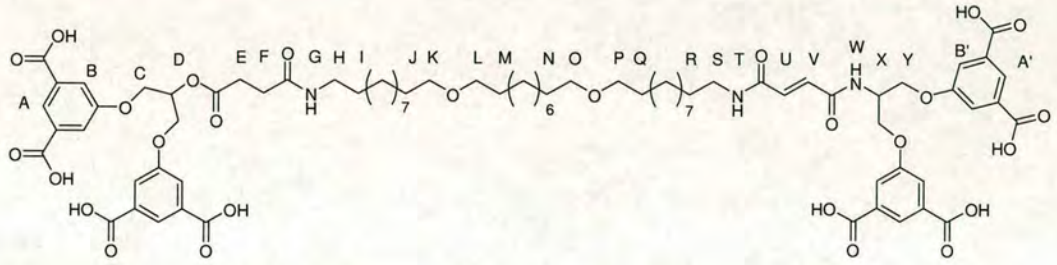
Preparation of {E}-N-[11-(10-{11-[3-(-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy -phenoxymethyl)-ethyl] ester (88).



A solution of **87** (0.2 g, 0.1 mmol) in CH_2Cl_2/TFA 9:1 (10 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and triturated twice with Et_2O and CH_2Cl_2 to give the product as colourless white solid. (150 mg, 99%); mp: 118-123 °C; ^1H NMR (400 MHz, CD_3OD:$CDCl_3$ 1:9): δ = 8.19 [m, 4H, Ar-H_A or Ar-$H_{A'}$], 7.75 [d, 4H, J = 1.3 Hz, Ar-H_B or Ar-$H_{B'}$], 7.72 [d, 4H, J = 1.3 Hz, CH_B or $CH_{B'}$], 6.24 [d, 1H, J = 13.0 Hz, CH_U or CH_V], 6.20 [d, 1H, J = 13.0 Hz, CH_U or CH_V], 5.52 [quin, 1H, J = 4.9 Hz, CH_D], 4.70 [quin, 1H, J = 5.0 Hz, CH_X], 4.38-4.23

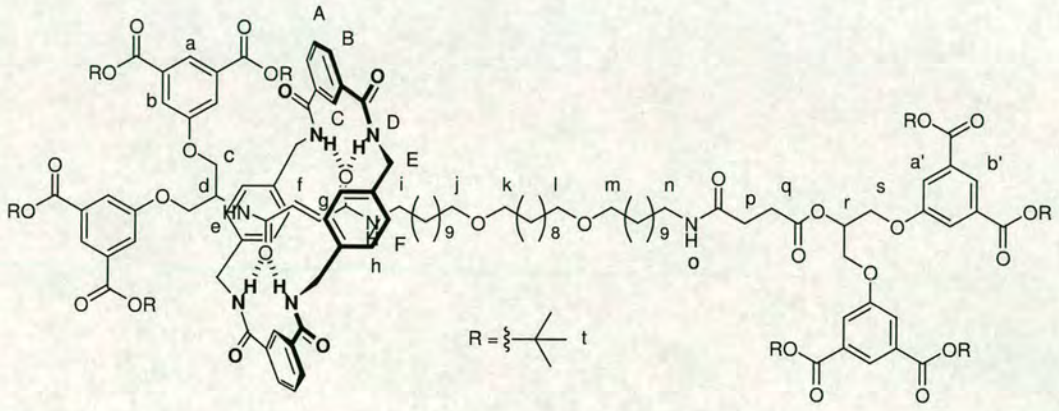
[m, 8H, CH_C and CH_Y], 3.31 [t, 8H, J = 6.5 Hz, CH_{KLOP}], 3.17 [t, 2H, J = 7.0 Hz, CH_S], 3.02 [t, 2H, J = 7.1 Hz, CH_H], 2.62 [t, 2H, J = 6.8 Hz, CH_E], 2.44 [t, 2H, J = 6.8 Hz, CH_F], 1.51-1.31 [m, 12H. CH_{IJMNQR}], 1.29-1.02 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0, 173.5, 168.5, 160.1, 160.0, 133.9, 133.8, 133.3, 133.0, 124.7, 121.0, 71.9, 71.7, 67.9, 67.8, 50.4, 41.0, 40.5, 31.4, 30.7, 30.6, 30.5, 30.4, 30.3, 30.2, 30.1, 29.8, 27.9, 27.2; FABMS: m/z = 1514 $[M+H]^+$; HRMS: m/z = 1514.9833 $[M+H]^+$ (anal. calcd. for $C_{78}H_{104}N_3O_{27}^+$: m/z = 1514.6857).

Preparation of {E}-N-[11-(10-{11-[3-(-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy -phenoxymethyl)-ethyl] ester (89).



A solution of **86** (0.2 g, 0.1 mmol) in CH_2Cl_2/TFA 9:1 (10 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and triturated twice with Et_2O and CH_2Cl_2 to give the product as colourless solid. (150 mg, 99%); mp: 149-152 °C; ^1H NMR (400 MHz, CD_3OD): δ = 8.18 [m, 4H, Ar-H_a and Ar-$H_{a'}$], 7.73 [m, 8H, Ar-H_b and Ar-$H_{b'}$], 6.92 [d, 1H, J = 15.2 Hz, CH_U or CH_W], 6.86 [d, 1H, J = 15.2 Hz, CH_U or CH_W], 5.53 [quin, 1H, J = 4.7 Hz, CH_D], 4.71 [m, 1H, CH_X], 4.40-4.18 [m, 8H, CH_C and CH_Y], 3.32 [bt, 8H, CH_{KLOP}], 3.19 [bt, 2H, J = 6.8 Hz, CH_T], 3.02 [t, 2H, J = 6.9 Hz, CH_H], 2.62 [t, 2H, J = 6.6 Hz, CH_E], 2.44 [t, 2H, J = 6.7 Hz, CH_F], 1.50-1.38 [m, 12H, CH_{IJMNQR}], 1.30-0.99 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100 MHz, d_7-DMF): δ = 172.3, 170.6, 166.6, 158.9, 158.8, 156.6, 133.8, 133.1, 132.3, 123.1, 119.4, 70.4, 70.1, 68.1, 67.2, 67.0, 39.2, 30.9, 29.8, 29.7, 29.6, 29.4, 27.4, 26.8, 26.2, 21.5; FABMS: m/z = 1514 $[M+H]^+$; HRMS: m/z = 1514.6897 $[M+H]^+$ (anal. calcd. for $C_{78}H_{104}N_3O_{27}^+$: m/z = 1514.6857).

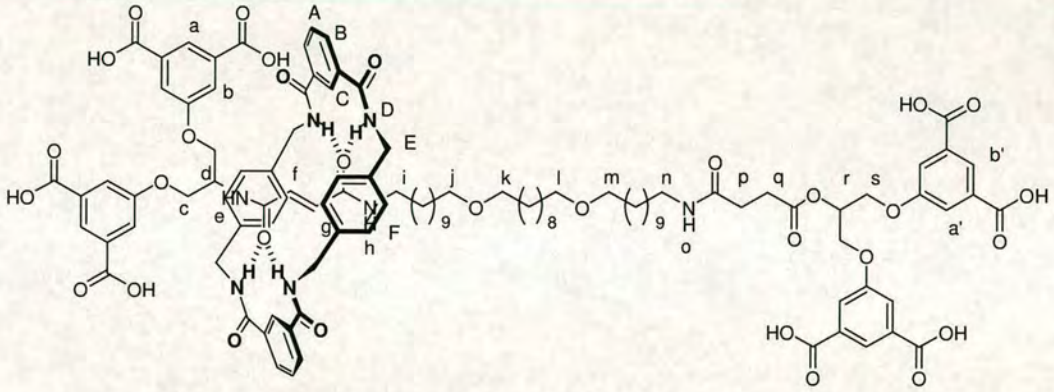
Preparation of [2]-(1,4,7,14,17,20-Hexaaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacoxane)-({E}-N-[11-(10-{11-[3-(-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester) rotaxane (90).



Thread **86** (490 mg, 0.25 mmol) and Et_3N (1.05 mL, 7.5 mmol) in anhydrous $CHCl_3$ (100mL) were stirred vigorously whilst solutions of para-xylylene diamine (510 mg, 3.75 mmol) in anhydrous $CHCl_3$ (20 mL) and isophthaloyl dichloride (760 mg, 3.75 mmol) in anhydrous $CHCl_3$ were simultaneously added over a period of 2 h using motor-driven syringe pumps. After 24 h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography (eluant: CH_2Cl_2/CH_3CN, 9:1) to furnish rotaxane **90** as colorless white solid. (530 mg, 85%); mp: 190-193 °C;^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.24 [bt, 2H, Ar-H_C], 8.18 [t, 2H, J = 1.3 Hz, Ar-H_a or Ar-$H_{a'}$], 8.11 [bt, 2H, Ar-H_a or Ar-$H_{a'}$], 8.05 [d, 4H, J = 7.5 Hz, Ar-H_B], 7.72 [m, 5H, NH_D and NH_h], 7.66 [dd, 8H, J = 1.2 Hz, J = 1.8 Hz, Ar-H_b and Ar-$H_{b'}$], 7.51 [t, 2H, J = 7.7 Hz, Ar-H_C], 7.10 [s, 8H, Ar-H_F], 7.06 [d, 1H, J = 3.0 Hz, NH_e], 5.95 [bt, 1H, NH_o], 5.76 [m, 2H, CH_f and CH_g], 5.51 [quin, 1H, J = 4.8 Hz, CH_r], 4.71 [m, 1H, CH_d], 4.59-4.14 [m, 16H, CH_E and CH_c and CH_s], 3.36 [m, 8H, CH_{jklm}], 3.17 [q, 2H, J = 6.5 Hz, CH_i], 3.06 [q, 2H, J = 6.3 Hz, CH_n], 2.69 [bt, 2H, CH_q], 2.41 [bt, 2H, CH_p], 1.59 [s, 36H, CH_t], 1.56-1.39

[m, 48H, CH_t and CH_2 alkyl], 1.35-1.14 [m, 40H, CH_2 alkyl]. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.8, 164.8, 164.6, 158.1, 157.9, 133.7, 133.5, 129.0, 123.5, 123.4, 119.3, 118.8, 81.9, 81.8, 71.0, 70.3, 66.2, 44.2, 39.8, 29.8, 29.6, 29.5, 29.42, 29.44, 29.3, 29.1, 28.1, 28.0, 26.9, 26.2. FABMS: *m/z* = 2496 $[M]^+$; HRMS: *m/z* = 2496.4009 $[M]^+$ (anal. calcd. for $C_{142}H_{197}N_7O_{31}^+$: *m/z* = 2496.4054).

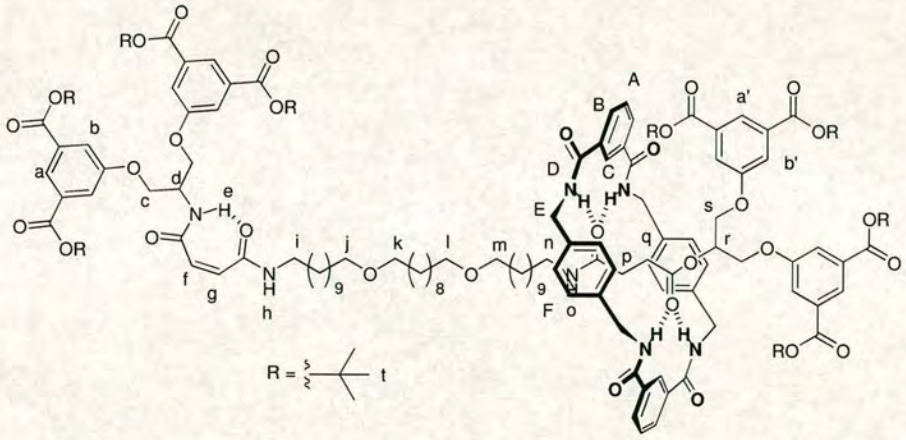
Preparation of [2]-(1,4,7,14,17,20-Hexaaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacoxane)-({E}-N-[11-(10-{11-[3-(-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy -phenoxymethyl)-ethyl] ester (91).



A solution of **90** (0.2 g, 0.08 mmol) in CH_2Cl_2/TFA 9:1 (10 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and triturated twice with diethyl ether and CH_2Cl_2 to give the product as colourless white solid. (163 mg, 99%); mp: 250 °C (decomp); ^1H NMR (400 MHz, CD_3OD): δ = 8.43 [bt, 2H, Ar-H_C], 8.05 [bt, 2H, Ar-H_a or Ar-$H_{a'}$], 8.03 [bt, 2H, Ar-H_a or Ar-$H_{a'}$], 7.88 [dd, 4H, *J* = 6.6 Hz, *J* = 1.1 Hz, Ar-H_B], 7.63 [d, 4H, *J* = 1.2 Hz, Ar-H_b or Ar-$H_{b'}$], 7.59 [d, 4H, *J* = 1.2 Hz, Ar-H_b or Ar-$H_{b'}$], 7.41 [t, 2H, *J* = 7.7 Hz, Ar-H_A], 6.92 [s, 8H, Ar-H_F], 5.90 [d, 1H, *J* = 15.0 Hz, CH_f or CH_g], 5.75 [d, 1H, *J* = 15.2 Hz, CH_f or CH_g], 5.40 [quin, 1H, *J* = 4.8 Hz, CH_r], 5.49 [quin, 1H, *J* = 4.7 Hz, CH_d], 4.39-4.08 [m, 16H, CH_E and CH_c and CH_s], 3.14 [m, 8H, CH_{jklm}], 2.99-2.81 [m, 4H, CH_i and CH_n], 2.48 [t, 2H, *J*=

6.7 Hz, CH_p], 2.29 [t, 2H, *J*= 6.6 Hz, CH_q], 1.41-1.24 [m, 12H, CH_2 alkyl], 1.15-0.89 [m, 40H, CH_2 alkyl]; ^{13}C NMR (100 MHz, CD_3OD): δ = 173.9, 173.5, 168.5, 168.4, 160.0, 159.9, 138.3, 135.0, 134.0, 133.8, 130.4, 129.0, 121.0, 120.8, 119.3, 71.9, 67.9, 45.0, 40.6, 30.75, 30.70, 30.66, 30.58, 30.45, 30.42, 30.32, 28.0, 27.3; FABMS: *m/z* = 2047 $[M]^+$; HRMS: *m/z* = 2047.9046 $[M]^+$ (anal. calcd. for $C_{110}H_{133}N_7O_{31}^+$: *m/z* = 2047.9046).

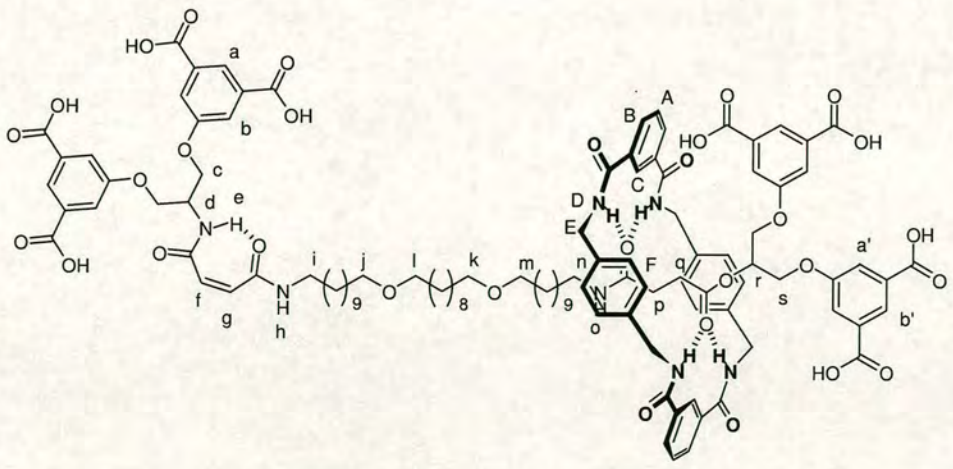
Preparation of [2]-(1,4,7,14,17,20-Hexaaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacoxane)-({Z}-N-[11-(10-{11-[3-(-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester) rotaxane (92).



The fumaramide rotaxane **90** (0.05 mmol) was dissolved in CH_2Cl_2 (30 mL) in a quartz vessel. The solution was directly irradiated at 254 nm usign a multilamp photo-reactor. The progress of the photoisomerization was monitored by TLC [CH_2Cl_2/MeOH (95/5)] or ^1H NMR. Different photostationary states were reached in a range of times not exceeding 20 min, after which the reaction mixture was concentrated under reduce pressure to afford the crude product. The crude material was purified by flash chromatography (eluant: CH_2Cl_2/CH_3CN, 90:10) to furnish compound **92** as yellow oil. Yield: 35%. Oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.39 [d, 1H, *J* = 7.8 Hz, NH_e], 8.31 [bt, 2H, Ar-H_C], 8.17 [bt, 4H, Ar-H_a or Ar-$H_{a'}$], 8.07

[dd, 4H, ^3J = 7.7 Hz, ^4J = 1.2 Hz, Ar-H_B], 7.89 [bt, 4H, NH_D], 7.69 [d, 4H, J = 1.3 Hz, Ar-H_b or Ar-$H_{b'}$], 7.67 [bt, 1H, NH], 7.55 [d, 4H, J = 1.2 Hz, Ar-H_b or Ar-$H_{b'}$], 7.47 [t, 2H, J = 7.7, Ar-H_A], 7.17 [s, 8H, Ar-H_F], 6.90 [bt, 1H, NH], 6.13 [bd, 2H, CH_f or CH_g], 5.35 [bquin, 1H, CH_r], 4.72 [bm, 1H, CH_d], 4.49 [dd, 4H, ^2J = 5.3 Hz, ^3J = 8.9 Hz, CH_E], 4.39-4.16 [m, 4H, CH_c], 4.05 [d, 4H, J = 5.3 Hz, CH_s], 3.37 [t, 8H, J = 6.7 Hz, CH_{jklm}], 3.27 [bq, 2H, CH_i], 2.86 [bq, 2H, CH_n], 1.59 [s, 36H, CH_t], 1.58 [s, 36H, CH_t], 1.56-1.40 [m, 14H, CH_p and CH_2 alkyl], 1.35-1.14 [m, 56H, CH_q and CH_2 alkyl]. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.0, 164.9, 164.7, 157.7, 141.0, 137.7, 134.3, 133.5, 133.4, 132.1, 130.7, 129.0, 128.8, 125.2, 119.2, 118.8, 82.2, 81.7, 70.9, 53.4, 44.2, 31.9, 31.4, 30.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.36, 29.31, 28.10, 26.9, 22.6; FABMS: m/z = 2496 $[M]^+$; HRMS: m/z = 2496.4004 $[(M)^+]$ (anal. calcd. for $C_{142}H_{197}N_7O_{31}^+$: m/z = 2496.4054).

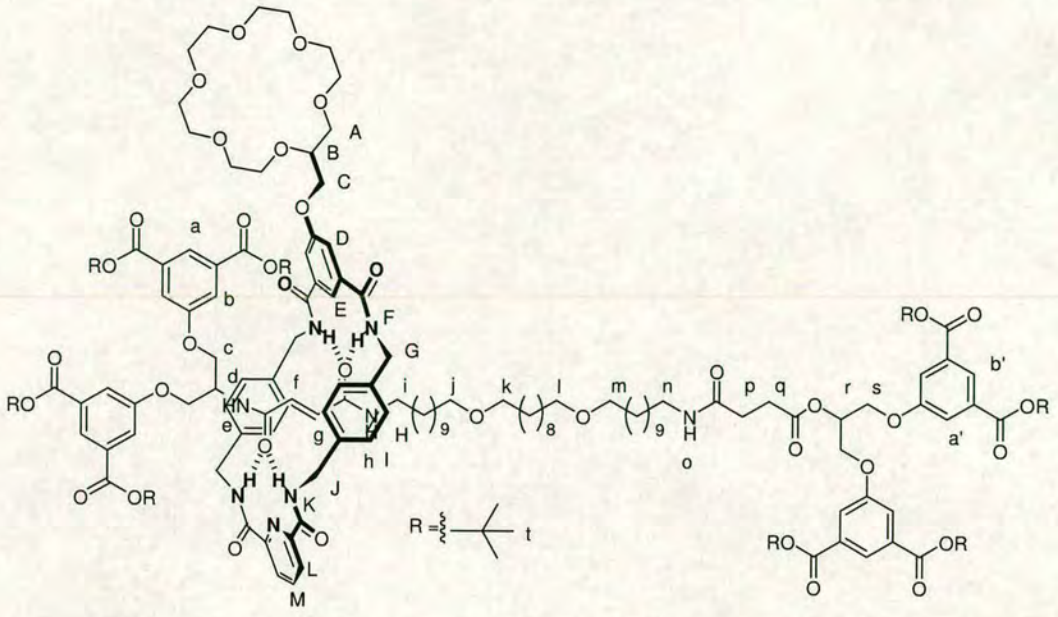
Preparation of [2]-(1,4,7,14,17,20-Hexaaza-2,6,15,19-tetraoxo-3,5,9,12,16,18,22,25-tetrabenzocyclohexacoxane)-({Z}-N-[11-(10-{11-[3-(-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy -phenoxymethyl)-ethyl] ester (93).



A solution of **92** (0.2 g, 0.08 mmol) in CH_2Cl_2/TFA 9:1 (10 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and triturated twice

with Et_2O and CH_2Cl_2 to give the product as colourless white solid. (163 mg, 99%); mp: 250 °C (decomp); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.18 [bt, 4H, Ar-H_a or Ar-$H_{a'}$], 8.02 [bt, 2H, Ar-H_C], 7.87 [d, 4H, *J* = 7.7 Ar-H_B], 7.72 [s, 4H, Ar-H_b or Ar-$H_{b'}$], 7.69 [s, 4H, Ar-H_b or Ar-$H_{b'}$], 7.44 [t, 2H, *J* = 7.7 Hz, Ar-H_A], 7.18 [s, 8H, Ar-H_F], 6.16 [d, 1H, *J* = 12.7 Hz, CH_f or CH_g], 6.01 [d, 1H, *J* = 12.7 Hz, CH_f or CH_g], 5.38 [quin, 1H, *J* = 4.4 Hz, CH_r], 4.61 [m, 1H, CH_d], 4.47-4.15 [m, 16H, CH_E and CH_c and CH_s], 3.20 [m, 8H, CH_{jklm}], 2.85-2.74 [m, 4H, CH_i and CH_n], 2.41 [t, 2H, *J* = 6.9 Hz, CH_q], 2.21 [t, 2H, *J*= 6.8 Hz, CH_p], 1.40-070 [m, 52H, CH_2 alkyl]. ^{13}C NMR (100 MHz, d_7-DMF): δ = 164.9, 164.5, 155.7, 152.6, 136.9, 136.8, 133.6, 131.4, 128.4, 127.5, 126.4, 119.4, 117.7, 68.8, 68.7, 65.4, 48.1, 41.4, 31.5, 30.1, 29.9, 28.8, 27.1, 25.8, 24.5, 24.4; FABMS: *m/z* = 2047 $[M]^+$; HRMS: *m/z* = 2047.9030 $[M]^+$ (anal. calcd. for $C_{110}H_{133}N_7O_{31}^+$: *m/z* = 2447.9046).

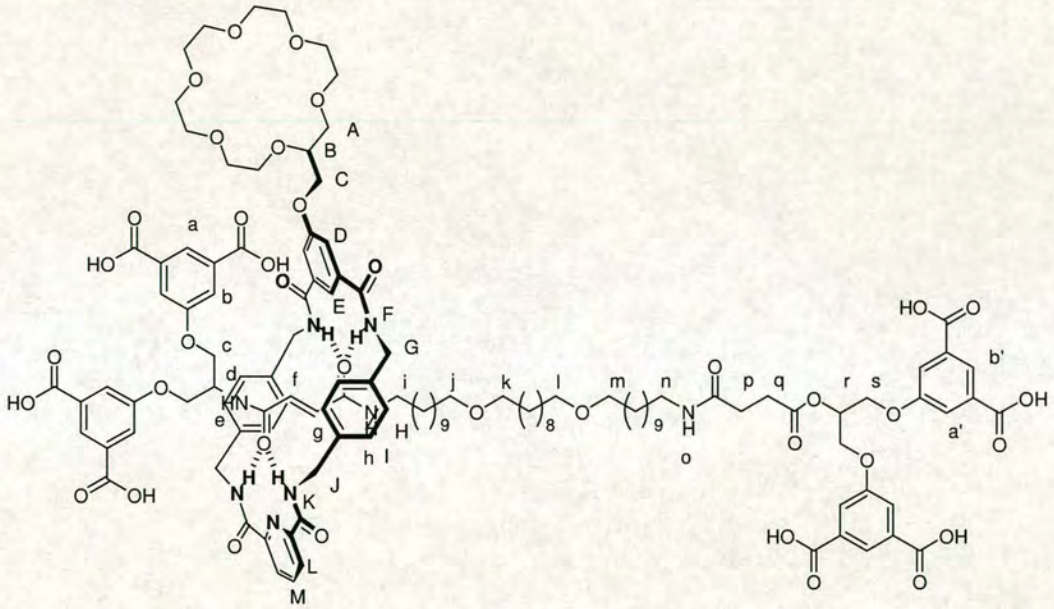
Preparation of [2](1,9,16,19,22-Pentaaza-2,8,17,21-tetraaoxo-5-(1,4,7,10,13,16-hexaoxa-Cyclooctadec-2-ylmethoxy)- -3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-({E}-N-[11-(10-{11-[3-(-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5 di-tert-butyl ester-phenoxy)1-1(3,5 di-tert-butyl ester-phenoxymethyl)-ethyl] ester) rotaxane (94).



Thread **86** (800 mg, 0.41 mmol) and Et_3N (0.36 mL, 2.50 mmol) in anhydrous $CHCl_3$ (100 mL) were stirred vigorously whilst solutions of **44** (500 mg, 1.23 mmol) in anhydrous $CHCl_3$ (20 mL) and **39A** (115 mg, 1.23 mmol) in anhydrous $CHCl_3$ were simultaneously added over a period of 2 h using motor-driven syringe pumps. After 24 h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography (eluant: CH_2Cl_2/MeOH, 9:1) to furnish rotaxane **94** as colorless oil. Yield: 15%. Oil. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.65 [bs, 2H, NH_K], 8.45 [bt, 1H, NH], 8.36 [d, 2H, J = 7.3 Hz, Ar-H_L], 8.20 [bt, 1H, NH], 8.18 [bt, 4H, Ar-H_a], 8.15 [s, 1H, Ar-H_{CE}], 8.02 [bt, 2H, J = 8.0 Hz, NH_F], 7.73 [bt, 2H, Ar-H_D], 7.66 [s, 8H, Ar-H_b], 7.49 [t, H, J = 7.8 Hz, Ar-H_M], 7.06 [bd, 1H, NH_e], 7.03 [m, 8H, Ar-H_F], 5.73 [m, 2H, CH_f and CH_g], 5.52 [m, 1H, CH_r], 4.80 [m, 1H, CH_d], 4.59-4.06 [m, 16H, CH_G and CH_J, CH_c and CH_s], 3.80 [m, 3H, CH_B and CH_C], 3.75-3.50 [m, 22H, CH_A and 18-Crown-6 CH_2], 3.36 [m, 8H, CH_{jklm}], 3.19 [m, 2H, CH_n], 2.98 [m, 2H, CH_i], 2.73 [m, 2H, CH_q], 2.47 [m, 2H, CH_p], 1.58 [s, 36H, CH_t],1.52 [s, 36H, CH_t], 1.35-1.09 [m, 52 H, alkyl CH_2]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 172.4, 170.9, 167.9, 166.3, 164.8, 164.5, 163.8, 158.1, 157.9, 149.2, 138.4, 134.2, 133.9, 133.7, 133.5, 131.1, 130.4, 129.2, 128.6, 127.8, 125.1, 123.6, 123.4, 119.2, 118.8, 81.8, 81.7, 71.0, 70.2, 66.2, 52.4, 44.2, 42.8, 40.2, 39.7, 31.0, 30.2, 29.8, 29.6, 29.5,6, 29.53, 29.51, 29.3, 28.1,

28.0, 27.0, 26.9, 26.2; FABMS: *m/z* = 2813 $[M+H+Na]^+$; HRMS : *m/z* = 2811.5363 $[M+H+Na]^+$ (anal. calcd. for $C_{154}H_{219}N_8NaO_{38}^+$ = 2811.5348).

Preparation of [2](1,9,16,19,22-Pentaaza-2,8,17,21-tetraaoxo-5-(1,4,7,10,13,16-hexaoxa-Cyclooctadec-2-ylmethoxy)- -3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-({E}-N-[11-(10-{11-[3-(-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy-phenoxymethyl)-acryloylamino]-undecyloxy}-decyloxy)-undecyl]-succinamic acid [-2-(3,5-carboxy-phenoxy)1-1(3,5-carboxy -phenoxymethyl)-ethyl] ester) rotaxane (95).



A solution of **94** (200 mg, 0.07 mmol) in CH_2Cl_2/TFA 9:1 (10 mL) was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and triturated twice with Et_2O and CH_2Cl_2 to give the product as colourless white solid. (164 mg, 98%) mp: 250 °C (decomp); ^1H NMR (400 MHz, CD_3OD): δ = 8.35-8.04 [m, 7H, Ar-H_L, Ar-H_a, Ar-H_E], 7.73 [bt, 2H, Ar-H_D], 7.77-7.63 [m, 8H, Ar-H_b], 7.53 [bt, 1H, Ar-H_M], 6.96 [m, 8H, Ar-H_H and Ar-H_I], 5.66 [d, 1H, J = 14.9 Hz, CH_f or CH_g], 5.56-5.43 [m, 2H, CH_f or CH_g and CH_r], 4.59-4.24 [m, 17H, CH_d, CH_G and CH_J, CH_c and CH_s], 4.20-4.09 [m, 3H, CH_B and CH_C], 3.77-3.45 [m, 22H, CH_A and 18-Crown-6

CH_2], 3.27 [m, 8H, CH_{jklm}], 3.14-2.94 [m, 4H, CH_n and CH_h], 2.60 [bt, 2H, CH_q], 2.41 [m, 2H, CH_p], 1.52-1.11 [m, 52 H, alkyl CH_2]; ^{13}C NMR (400 MHz, d_7-DMSO): δ =168.4, 168.3, 139.7, 135.0, 134.6, 134.0, 133.8, 131.8, 131.6, 130.6, 130.0, 129.9, 128.4, 128.2, 124.8, 120.9, 120.7, 71.8, 71.7, 71.1, 67.9, 66.9, 52.9, 43.4, 40.5, 31.4, 30.7, 30.6, 30.5, 30.4, 30.2, 29.5, 28.1, 28.0, 27.3; FABMS: *m/z* = 2364 $[M+Na]^+$; HRMS : *m/z* = 2364.0378 $[M+Na]^+$ (anal. calcd. for $C_{122}H_{156}N_8NaO_{38}^+$) : *m/z* = 2364.0418).

[1] S. R. Chhabra, A. Mahajan, W. C. Chan, *J. Org. Chem.* **2002**, *67*, 4017.

[2] L. J. J. Hronowski, W. A. Szarek, G. W. Hay, A. Krebs, W. T. Depew, *Carbohydrate res.* **1989**, *190*, 203.

[3] H. Ihara, M. Takafuji, C. Hirayama, D. F. O'Brien, *Langmuir* **1992**, *8*, 1548.

[4] A. Leydet, V. Barragan, B. Boyer, J-L Montéro, J-P Roque, M. Witvrouw, J. Este, R. Snoeck, G. Andrei, E. De Clercq, *J. Med. Chem.* **1997**, *40*, 346.

4 Single Molecule Experiments on a Surface Attachable Bistable Molecular Shuttle (SABMS)

4.1 Introduction to Atomic Force Microscopy.

Atomic Force Microscopy (AFM)[1] belongs to the family of scanned-proximity microscopes. Different from its main predecessor, Scanning Tunneling Microscopy (STM), AFM is able to image conducting and nonconducting samples under near-physiological conditions. AFM operates by measuring attractive or repulsive forces between a tip at the end of a cantilever and the surface atoms as the tip is scanned over the surface of the sample (Figure 1a). The mode of action is reminiscent to the way in which a record player works (Figure 1b).

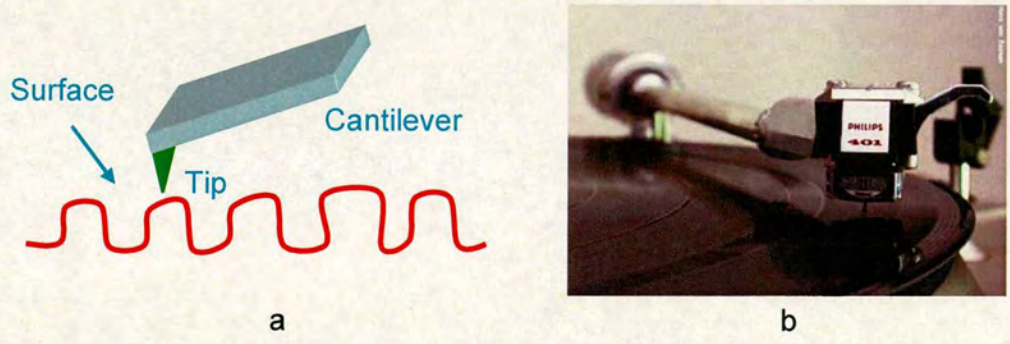


Figure 1. a) an AFM tip is scanned over the surface, b) a record player operates similarly to AFM.

The first AFM instrument was used to examine insulating surfaces. It was invented by Gerd Binnig and Christoph Gerber (Nobel Prize in Physics in 1986).[2, 3] In their device, the tip was made of a tiny diamond chip and the cantilever was a strip of gold foil. The tip at the end of the cantilever was pressed against a surface while the sample was scanned beneath the tip. The up and down movement of the cantilever produced changes in the tunnelling current passing between the cantilever and a second tip that is positioned above the cantilever. The result was a map of the surface with a resolution of about 300 Å.

Since the forces between the tip and the sample are of femto Newton (fN) magnitude, direct measurement of these forces is an extremely difficult task. To overcome this problem tunnelling current devices have been replaced with optical detectors. As the AFM tip probes the surface by moving along its contours, the movement of the cantilever is detected with a laser beam focused on the head of the cantilever which is then reflected onto a photodetector consisting of adjacent photo diodes. The movement of the cantilever causes a two fold angular deflection of the laser beam and an image is generated dependent on the difference between the two photodiodes. The photodetector measures the differences between the two beams that strike the diodes and this data is sent as change in voltage to a piezo-electric transducer placed underneath the sample, which in turn is capable of sub angstrom resolution measurements in all directions. This is known as electronic feedback (Figure 2).

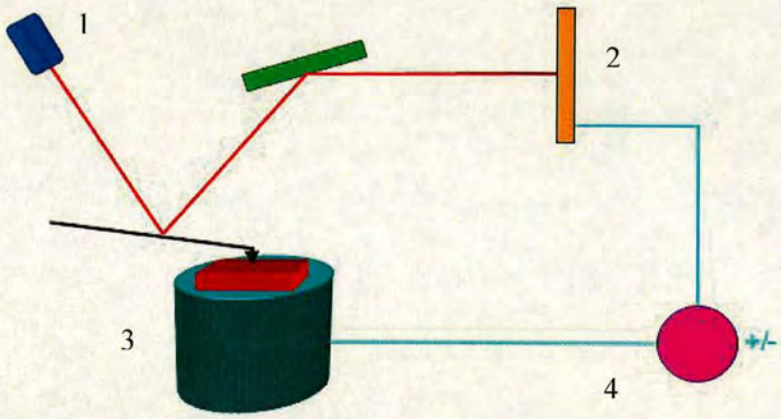


Figure 2. How an optical lever operates. The distance between the cantilever and detector is normally about three orders of magnitude greater in length than the cantilever, thus minimal motions of the cantilever are greatly magnified. Components: 1) laser, 2) photodiodes, 3) piezo-electric transducer, 4) photodetector.

AFM operates in two modes in which the electronic feedback is either switched on or off. In the first mode the piezo-electric transducer maintains a constant force between tip and sample, moving the latter up and down in response to a change in force. This mode is used to obtain height information. In the other mode when the piezo-electric transducer is switched off, the sample is maintained at a constant height, yielding force information.

4.1.1 AFM Modes.

One of the main uses of AFM is in generating topographic imagery of surfaces. AFM is based on three imaging modes (Figure 3): a) contact mode; b) non contact mode; c) tapping mode. These are discussed in the following sections.

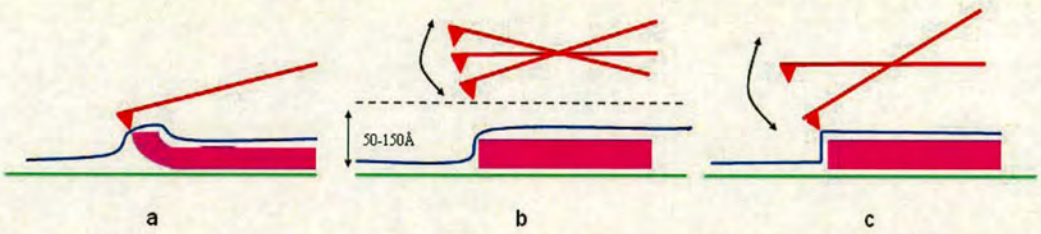


Figure 3. AFM modes. a) Contact mode, b) non contact mode, c) tapping mode.

4.1.1.1 Contact Mode.

In Contact Mode, the tip is moved across the sample surface while maintaining the deflection of the cantilever by restoring the height of the sample (Figure 3a). In this method low repulsive forces with a mean value of 10^{-9} N are measured. Contact mode AFM works via an electronic feedback switch responsible for maintaining constant deflection of the sample by changing the relative position of the tip (lowering or raising the sample) and, therefore, the motion along the z axis corresponds to the sample topography. One of the limiting applications of contact mode AFM is that either sample or tip can be damaged by dragging the tip across the sample. Damage is due to lateral tracking forces between tip and sample. Contact mode AFM can operate in UHV or ambient atmosphere but most of the measurements are performed in liquid medium. One of the main problems of contact mode AFM under ambient conditions is the formation of moisture layers and nitrogen over the sample which may be on the order of 10-30 monolayers thick. When the tip touches the layer a meniscus is formed and the cantilever is subjected to attractive forces on the order of 100 nano Newton (nN) (the size of the forces depends also on the tip geometry). The use of liquids eliminates such capillary forces,

and reduces Van der Waals interactions, and allows the measurement of biologically important processes. However, in some cases it might not be appropriate in liquid medium.

4.1.1.2 Non Contact Mode.

In non contact mode AFM the tip is kept 50-150 Å above the sample surface (Figure 3b). In this method Van der Waals and other long range forces are detected. Compared with the repulsive forces encountered in the contact mode, these forces tend to be particularly small. To facilitate the measurement of these weak forces the cantilever is oscillated with a known frequency so any change in amplitude is detected and used to generate the topographic image of the sample. Non contact mode AFM can be performed either with constant amplitude or constant resonance frequency in the presence or absence of electronic feedback. One of the main limitations of this method is the small signal from Van der Waals forces which may be overcome often times by strong interaction between the tip and thicker fluid contaminant layers.

4.1.1.3 Tapping mode.

Tapping mode AFM is one of the most advanced modes as it overcomes most of the problems seen with the previous two modes. Tapping mode operates similar to the non contact mode with some variations. In this method the tip is made to oscillate and is placed in contact with the sample surface for a short period during each oscillation cycle and then lifted to avoid dragging across the sample surface (Figure 3c). Changes in the amplitude are used to measure surface structures. In tapping mode AFM the oscillating frequency of the tip-cantilever is quite rapid and as such it allows for reduction of the adhesion forces between tip and surface by preventing the tip from sticking to the sample surface.

4.1.1 AFM and Biology.

The past 20 years has seen tremendous improvements in AFM utility, and the technique has brought an enormous amount of insight to all areas of science. While the scope of this chapter is not the use of AFM as a tool for imaging surfaces in atomic resolution, there is one example which deserves consideration here. This example is in connection with the previous chapter where ion transporters are discussed. Alzheimer's, Parkinson's, and Huntington's diseases, diabetes and tuberculosis are all diseases connected by some degree to protein misfolding, which in turn causes nanometer-sized pores to form in brain nerve cells by pathological plaques. Through these pores, ions like Ca^{2+} may enter which disrupts cell homeostasis. By using AFM, Lal and coworkers were able to obtain images of such ion channels and their work was instrumental in understanding the cause of these problems[4] Other recent examples demonstrating the scope and broad utility of AFM are found in the study of the elasticity of polymers by measuring the adhesion forces when the tip is pushed into the surface of the sample and then retracted[5-6] or in building Boolean circuits by exploiting enzymatic cleavage reactions on DNA.[7]

4.2 Single-Molecule Force Spectroscopy.

One such recent application of AFM with particular relevance to the current work is Chemical Force Microscopy (CFM).[8, 9] Here the AFM tip is functionalized with particular chemical species by formation of a Self Assembled Monolayer (SAM) over its surface. The functionalized tip is scanned over the sample (often also an appropriate and complimentary SAM), and differences in interaction forces between the two are detected. This technique may also be extended to Single-Molecule Force Spectroscopy (SMFS) wherein CFM is used to obtain information about inter- and/or intramolecular forces at the level of individual molecules (Figure 4).

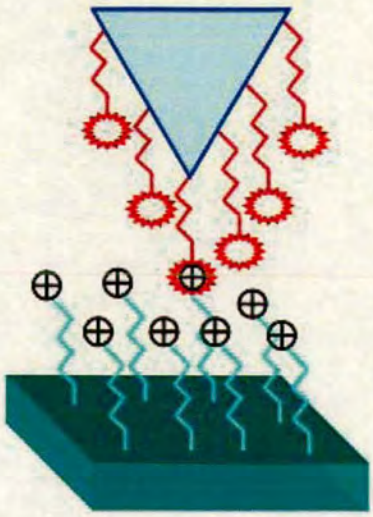


Figure 4. Chemical Force Microscopy.

Over the past decade SMFS techniques have been successfully used to investigate several molecular systems. The first example of SMFS was reported in 1994 by Gaub and coworkers.[10] In this study an AFM tip was coated with a receptor (biotin) and brought into contact with a sample coated with the appropriate ligand (streptavidin), and then the two were pulled apart. This method allowed measurement of the forces involved in a single binding event between biotin and streptavidin. Other examples of the utility of SMFS are found in its use to measure the binding of single metal-protein complexes,[11] to discriminate between chiral molecules,[12] in the study of cation binding processes,[13] in the measurement of bimolecular potential energy surfaces,[14] in ion binding between cations and SAMs,[15] in discrimination between antioxidant and UV-light stabilizers on polymer surface,[16] and also in probing enzyme orientation on charged surfaces.[17] A recent particularly interesting example of SMFS was reported by Mattay and coworkers[18] wherein they investigated a photochemical single molecule switch using Resorc[4]arene.

Gaub and coworkers have summarised the important molecular characteristics into a useful diagram (Figure 5), which illustrates the accessible force window of SMFS.[19] This window makes it possible to see at a glance a whole range of interactions from entropic forces at several fN[20] to the rupture of covalent bonds at a few nN.[21]

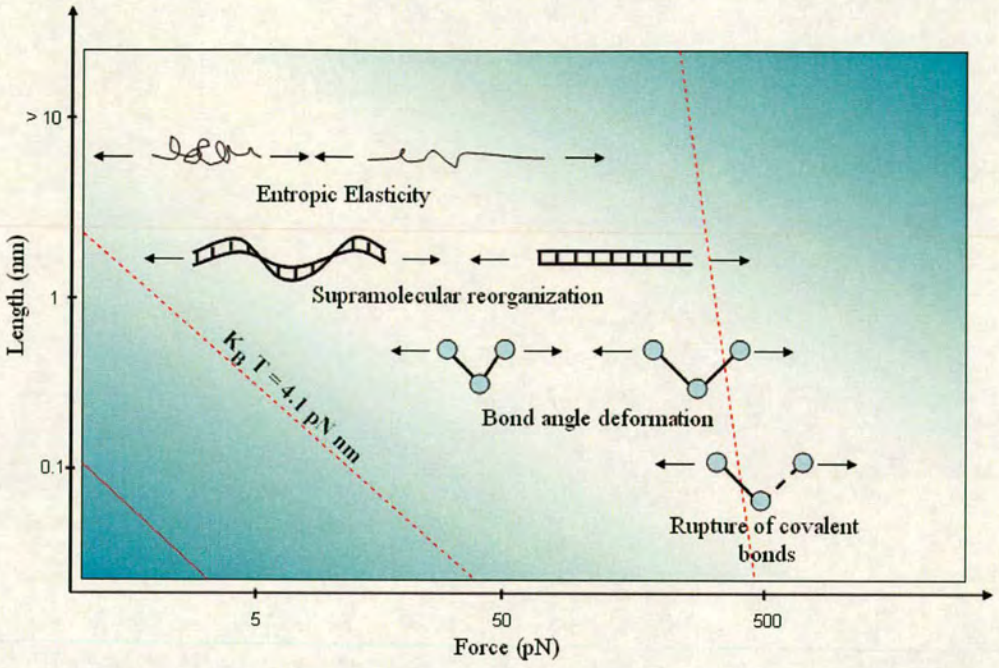


Figure 5. Typical forces and length scales (both scales are logarithmic) in SMFS. The experimental accessibility of mechanical information is limited to the dotted lines on the plot. The shaded area in the lower left corner indicates the region of limited thermal stability of molecular structures (length multiplied by force = thermal energy, k_BT = 4.1 pN nm at room temperature). The upper limit to the accessible experimental force range is determined by the rupture of covalent bonds at several nanonewton. [19]

Previously, we discussed the fascinating world of molecular machines and mentioned that an important utility of these systems is in mimicking some functional aspects of living organisms and in doing so, constantly improving, realizing and developing new biomimetic materials. Many synthetic molecular machines are designed to perform a specific task, a chemical change, in response to external stimuli. One of the current questions that has arisen from progress in this area is how can external stimuli (such light or electrons or pH) be transformed into mechanical force at the single molecular level, and vice versa? We attempt to answer this question with the work presented here.

4.2.1 Single Molecule Force Spectroscopy on Molecular Machines.

'Molecular machines' are a subset of 'molecular devices' (functional molecular systems) in which some stimulus triggers the controlled, large amplitude or directional mechanical motion of one component relative to another (or of a substrate relative to the machine) which results in a net task being performed.[22] If the system in question is able to return to its initial state, by making use of reversible molecular processes, it may (theoretically) be capable of turning external energy into mechanical work at a single molecule level. In order to illustrate this point, consider a reversibly switchable polymer chain which is capable of changing between a short form and an extended form. This system exhibits an operational cycle based on the two states of the polymer.[23] The forces involved with such a system are shown in Figure 6a where a polymer exhibits a reversible transition from a short to an extended conformation. For simplicity the polymer is represented as an extendable band (Figure 6b) which is able to lift a weight attached to one end.

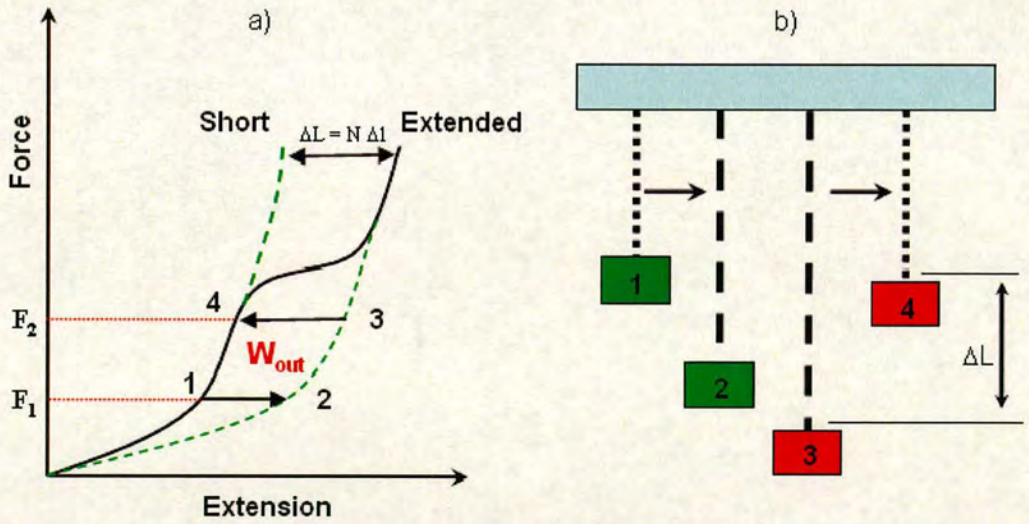


Figure 6. a) Force-Extension curve and b) a model capable of converting energy at the molecular level.

The cycle starts at point **1** where the polymer is fully contracted and is in its natural short conformation. At point **2** the polymer is transformed to an extended

conformation as a result of some external stimulus or chemical modification. At this point an external load is attached to the polymer (in Figure 6b, the weight attached to the polymer is indicated by a colour change from green to red), and, as illustrated in the force-extension curve, extends the polymer conformation due to the additional load (point **3**). If the system is now converted back to its starting state, by a second stimulus or chemical modification, it contracts to a short conformation illustrated as point **4** of the curve. At this point the system has done work – a mass has been moved a distance Δl. The load may then be removed (colour change from red to green) and the system returned to the starting point of the cycle. The chemical contraction of the polymer, $\Delta = N \cdot \Delta l$, from an extended to a short conformation provides the energy needed for performing the mechanical work (which is the sum of the energy of the external stimulus plus the energy of the load). The mechanical work of the cycle is equal to the area under the curve.

4.2.1.1 Single Molecule Force Spectroscopy on polymers.

Direct measurement of the forces involved in the preceding section may be obtained by AFM. In this experiment, a polymer chain is attached on one end to a rigid surface and the other end to a cantilever spring of an Atomic Force Microscope. When the polymer is fully "relaxed" there is little restriction to its movement and it may adopt all possible configurations. Stretching the polymer decreases the number of available configurations and work must now be done to overcome entropic differences between the short (higher entropy) and extended (lower entropy) configurations. The "load" here is the force applied by the AFM cantilever. The key and unique factor now is the chemical transformation which provides the system with the ability to switch back to its original conformation (from stiff to flexible if the reverse process is performed by the AFM applied force). Gaub and coworkers have demonstrated this process with light powered molecular machines based on poly(azobenzene peptides). They showed that the contour length of polymers chains was selectively changed by isomerizeing an azobenzene moiety from a *cis* to *trans* conformation using light as external stimulus.[24, 25] Vancso and coworkers[26] also proposed a redox switchable polymer based on poly(ferrocenyldimethylsilane) (PFS).

This mechanism behind their system is the two available redox states of the PFS moiety. The oxidized form is positively charged and leads to a stiffening of the molecule (extended form) while the reduced form is neutral and represents the short form.

4.2.2 First example of SMFS on a switchable bistable molecular shuttle. Analysis of Stoddart's model.

Stoddart and coworkers have prepared and studied a system that closely resembles the one presented by ourselves. Their prototypical system employs a bistable molecular shuttle, a [2]rotaxane where the shuttling of the ring along the thread is controlled electrochemically at one of the stations.[27, 28] In this work Stoddart and coworkers have described[29] a method used to measure the power stroke of the bistable rotaxane.

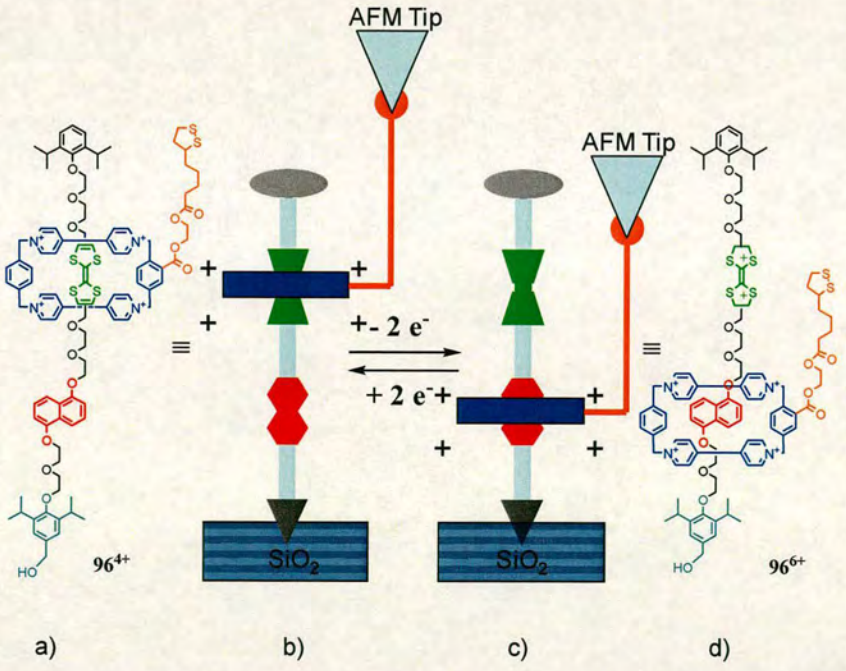


Figure 7. a) and d) Structures of bistable molecular shuttles used in SFMS experiments. The $CBPQT^{4+}$ ring (light blue), redox controllable TTF station (dark red), and the electron rich DNP station (red).are indicated; b and d) schematics of AFM of rotaxane **96**.

In this experiment, a gold AFM tip is attached to the macrocycle of the [2]rotaxane through a thioctic acid tethered moiety and a hydroxymethyl group on one of the

stoppers provides the attachment to a monolayer on a SiO_2 surface (Figure 7). This modified rotaxane allows the use of AFM in measuring steric and electrostatic interactions between the macrocycle (a cyclobis(paraquat-p-phenylene) $CBPQT^{4+}$) and the two stations on the thread (an electron rich dioxynaphthalene (DNP) station and a redox controllable tetrathiafulvalene station (TTF)). When the TTF station is reduced, $CBPQT^{4+}$ displays a stronger interaction with it compared to the DNP station, and therefore resides predominantly over this station. However, when the TTF station is oxidized to TTF^{2+} the $CBPQT^{4+}$ ring is repulsed by the charges of the TTF^{2+} station, and shuttling of the ring to the DNP station results. The system returns to its initial state upon chemical reduction of the TTF^{2+} to TTF. The repulsion between $CBPQT^{4+}$ and TTF^{2+}, responsible for destabilizing the macrocycle and forcing it to shuttle to the other station, corresponds to a repulsive energy on the order of 65 (kcal mol^{-1}). This is a good example of a nanoscale linear molecular machine capable of reversibly expanding and contracting in the presence of an external stimulus – in this case an oxidant or a reductant, respectively.

4.2.3 Single Molecule Force Spectroscopy (SMFS) on a Bistable Hydrogen-Bonded Molecular Shuttle.

We now describe the use of SFMS to measure the binding forces involved in our bistable molecular shuttle. Our model utilizes a shuttling mechanism well known to our group[30] based on a fumaramide-maleamide switching moiety and a succinamide-ester functional group.[31] In this experiment we intend initially to use SMFS to measure the strength of the four hydrogen bonds between the fumaramide template and the tetra amide macrocycle by forcing the macrocycle away from this station mechanically. The reverse situation where the macrocycle is mechanically removed from the succinic station will then be explored. The combination of the two results will hopefully shed some insight on the extent of mechanical work that may be expected from the entire cycle. This work should add and enhance an expanding body of current knowledge since similar SMFS studies have already been performed to measure hydrogen bond strength. Gaub and coworkers[32] were able to measure base pair-unbinding forces of G-C and A-T nucleotides in DNA. In another example

Ratler and coworkers were able to perform similar studies on nucleotides.[33] In addition, SMFS has also recently been used to investigate the hydrogen bonding in peptide mimetic β-Sheet motif,[34] to study modular polymer constructed with strong hydrogen bond units[35] and also to directly examine the propensity of polysaccharides to form inter-residue hydrogen bonds under various solvent conditions.[36]

4.2.3.1 Structure of the Surface Attachable Bistable Molecular Shuttle.

To allow single molecule manipulation the Surface Attachable Bistable Molecular Shuttle (SABMS) **109** must have the following features (illustrated in Figure 8a): a) two points of attachment; b) a long spacer between the two stations - long enough to allow measurement of the shuttling process; and c) a long linker used to connect the AFM tip and the [2]rotaxane needed in order to minimise the formation of unspecific adhesion forces between the AFM tip and the SABMS.

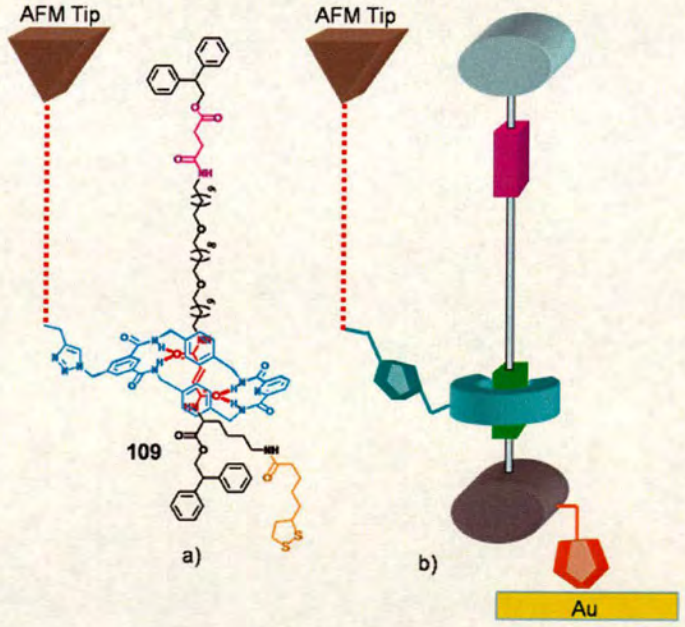


Figure 8. a) Structure of Leigh Surface Attachable Molecular Shuttle used in SMFS experiments; the tetra amide macrocycle covalently bound to the PEG segment (light blue), fumaramide station (green), thioctic tethered acid moiety (orange), succinic amide-ester station (purple), PEG segment covalently bound on one end to the macrocycle and attached to a silicon nitride AFM tip on the other end (red); b) schematics of SMFS on **109**.

The distance between the two stations is about 40 Å and consists of a long alkyl chain made of 32 atoms of carbon with two oxygen ether linkages. By comparison, the length in previous bistable molecular shuttles is about 15 Å and is not thought to be enough to accurately study shuttling using the AFM technique. The linkage between the AFM tip and the macrocycle is a Poly(ethylene-glycol) (PEG) segment of about 350 Å in length when in a fully stretched conformation. One end of the PEG segment is covalently bound to the ring of the [2]rotaxane via the triazole moiety and the other end is attached to a silicon nitride AFM tip by indentation. One thioctic acid tethered moiety on one of the stoppers provides the attachment to the monolayer on gold surface (Figure 8b). The system is embedded in a Self Assembled Monolayer (SAM) made up of short alkyl chains (12 methylenic groups).

4.2.3.2 How a Surface Attachable Bistable Molecular Shuttle works.

The first stage of the experiment will make use of Single-Force Molecule Spectroscopy (SFMS) to measure the strength of the four bifurcated hydrogen bonds between the fumaramide template and the tetra amide macrocycle as mentioned above. Here the macrocycle, which is strongly held around the fumaric station, will be forced away to the succinic side of the thread by lifting up the AFM cantilever. Initially, retraction of the AFM tip will stretch the PEG linker until it reaches its extended conformation, followed by movement of the macrocycle. The movement ceases when the stopper next to the succinic amide-ester station prevents the dethreading of the macrocycle. Figure 9 illustrates the process.

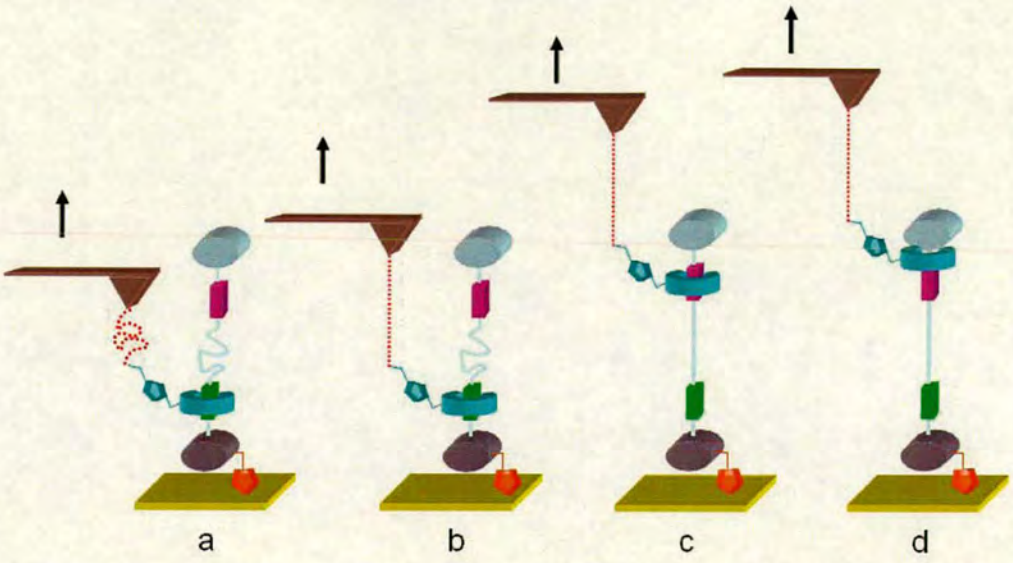


Figure 9. Schematics of AFM force spectroscopy on the SABMS with the olefin in a *trans* conformation (green station). **a)** Initial state where the PEG linker and the thread adopt a random coil conformation and the ring is over the fumaramide station. **b)** Retraction of the AFM tip stretches the PEG linker until its maximum length. **c)** Once the linker is completely stretched the AFM tip should start pulling the macrocycle off the fumaramide station. The macrocycle, which is initially held by the four strong hydrogen bonds is forced to move to the other station, and should be observed by an easing of the force applied by the AFM. Shuttling to the succinic amide-ester station (purple station) also stretches the thread until the macrocycle resides over the succinic station. **d)** At this point the AFM cantilever should record an increased force because one of the bulky stoppers is mechanically preventing the dethreading of the macrocycle and further stretching.

Irradiation of the fumaramide moiety at 254 nm produces the corresponding *cis* maleamide rotaxane resulting in a considerable reduction of the binding strength between the macrocycle and the new maleic station. The succinic amide-ester station is now the preferred binding site for the macrocycle. Repeating the above AFM experiment on this new system completes the study of the forces involved in shuttling of this system. The SFMS on the *Z*-rotaxane provides a baseline system from which to compare interactions of the macrocycle and thread in the previous process, wherein the ring is strongly held in the proximity of the fumaramide station of the *E*-rotaxane. The AFM tip, when pulled back, will stretch the PEG linker and

the thread until the system reaches its extended conformation, however, in this instance, shuttling of the macrocycle from one station will not take place as the macrocycle is already held by the station furthest from surface attachment – ie the system is fully extended. If a stimulus is applied to change the properties of the station nearest to the surface attachment back to its original state (*E* conformation) the ring will have a tendency to shuttle back to the green station and bend the AFM cantilever downward in the process. Figure 10 shows the complete cycle of this experiment.

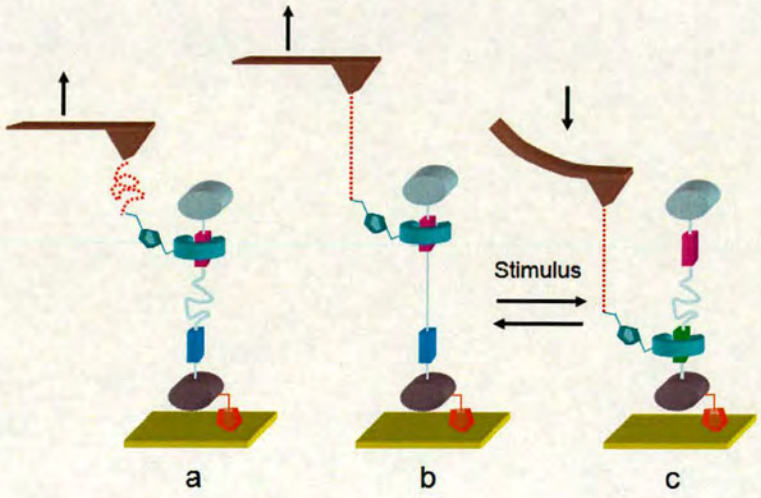


Figure 10. Schematics of AFM force spectroscopy on the SABMS with the olefin in *cis* geometry (light blue, maleamide station). **a)** Initial state where both PEG linker and thread adopt a random coil conformation and the ring is over the succinic amide-ester station (purple station). **b)** Retraction of the AFM tips strengthens the PEG linker and the thread until the system reaches its maximum length. **c)** Upon isomerization the AFM cantilever records a downward force exerted by the shuttling of the macrocycle to the most stable station (green fumaramide station). The force required for the shuttling is the energy needed for performing the mechanical work of the entire cycle.

In short, the purpose of this work is to first determine the force needed to translocate the macrocycle away from the preferred *trans* station and, subsequently, to measure the forces involved in the opposite process when station is isomerized from the *cis* to the *trans* and the affinity of the macrocycle for this station is restored. These two experiments should provide data on the energy needed to perform the entire cycle.

4.2.3.3 Importance of the PEG linker.

The architecture of the system and single attachment of the PEG linker is one of the most important features of our Surface Attachable Bistable Molecular Shuttle (SABMS). To enable single molecule manipulation only one PEG segment molecule per system must be attached to the AFM tip during the measurements. The PEG segment is covalently attached to the macrocycle of the SABMS on only one end and the other one is available for attachment to the AFM tip. Attachment to the AFM tip is performed by indenting the tip into the polymer layer to allow individual PEG segments to be picked up by adsorption. To avoid multiple attachments the tip is moved up and down until only one PEG segment is attached to the tip. Figure 11 shows an example of a force-extension diagram of a PEG segment in work by Gaub and coworkers.[37]

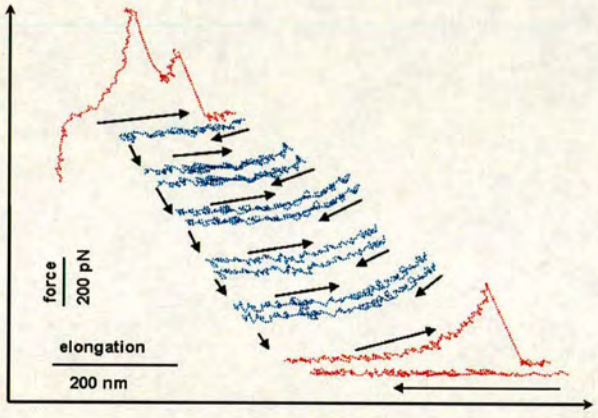


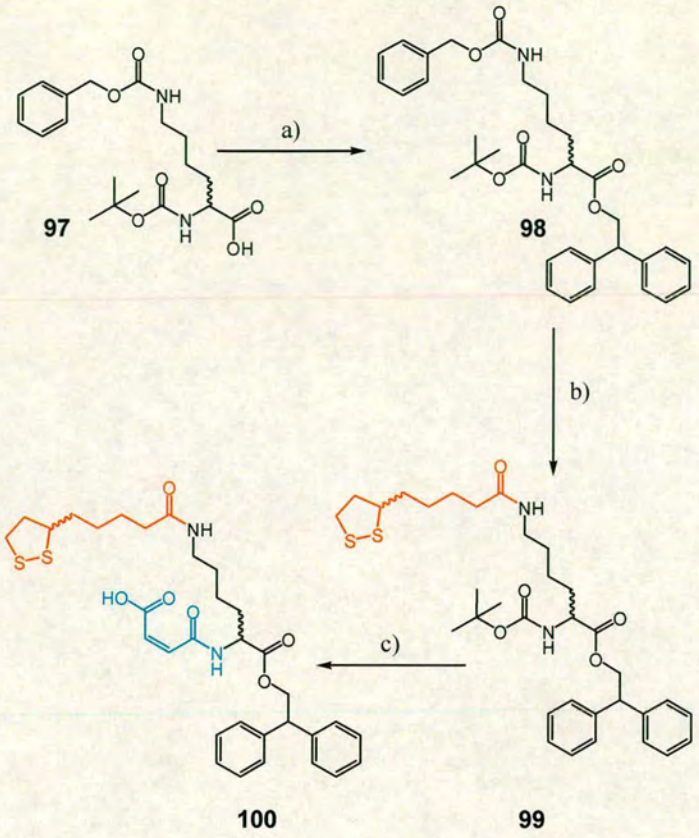
Figure 11. Reversible extension of a PEG segment. At first contact three molecules had attached to the tip. During the first extension two of them ruptured or detached. The blue lines show the repeated stretching of one remaining PEG segment. In the last scan the force was increased beyond rupture.

The PEG fragment, due to its flexibility, is incorporated into the experiment to reduce non specific interactions between the AFM tip and the SABMS.[38, 39] Recent work by Joselevich and coworkers[40] showed that PEG can effectively convert the configurational constraint imposed by single molecular recognition events between a Streptavidin ligand and a Biotin receptor into a measurable force.

4.3 Synthesis of the thread.

4.3.1 Synthesis of the Maleic Station.

To act as a stopper and contain the macrocycle in the system, the maleic station bears two bulky phenyl groups which mechanically prevent the dethreading of the macrocycle. The core molecule of the maleic station is Lysine. This commercially available amino acid allows one to exploit both of the primary amines for the design of the station. Lysine with primary amines orthogonally protected with Cbz and Boc was coupled with the diphenyl stopper using EDCI and DMAP to give ester **98** in 92% yield (Scheme 1, step a). The Cbz group was removed using H_2 in the presence of catalytic amount of Pd/C in THF and this fragment was further coupled with Lipoic acid to yield amide **99** in 93% yield (Scheme 1, step b). This thioctic acid tethered moiety has been successfully employed previously for attachment to gold surfaces, for example in bistable molecular shuttles[41, 42] and in other systems such as cyclodextrin[43] and tetraammine ruthenium complexes.[44] The Boc group was removed using TFA in CH_2Cl_2 and the resulting free amine reacted with maleic anhydride in THF to yield **100** in 90% yield (Scheme 1, step c).

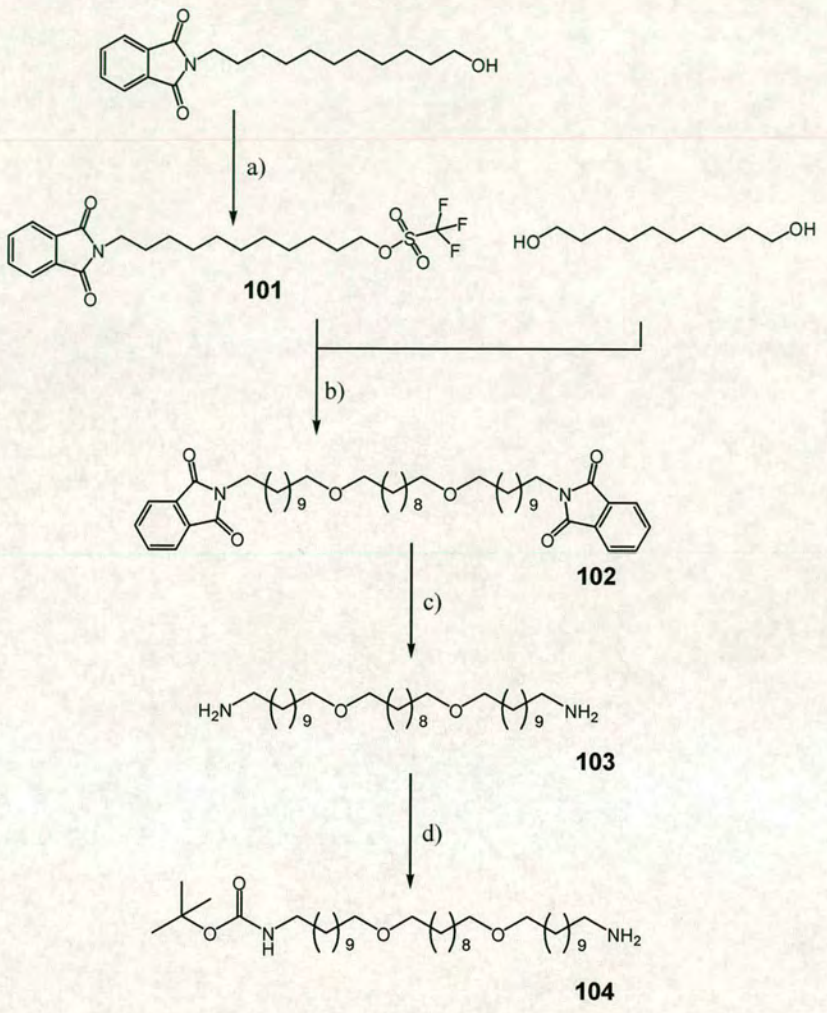


Scheme 1. Synthesis of the Maleic Station **100**. a) EDCI•HCl, DMAP, 2,2-diphenyl ethanol, CH_2Cl_2, 92%; b) H_2, Pd/C (10%), THF, then EDCI HCl, 4-DMAP, (D/L)-Lipoic acid, Et_3N, CH_2Cl_2, 93%; c) TFA/ CH_2Cl_2 (1:9), then maleic anhydride, Et_3N, THF, 90%.

4.3.2 Synthesis of the spacer.

The spacer needs to have an NH_2 group on either end to allow for coupling with the two different stations. One of the amines is protected with a Boc group and the other one is left free. Fragments of the spacer were coupled together by following the procedure by Springer and coworkers[45] and Anderson and coworkers[46] in Chapter 3. Although the synthesis of the required spacer has already been reported in chapter 3 an improved preparation has been developed by us and will be described here. A spacer bearing two identical protecting groups is prepared first. A triflate activated alcohol **101** with a phthalimide protected primary amine is reacted with decanediol to give **102** in 71% yield (along with the formation of the mono- addition product) (Scheme 2, step b). Removal of the two phthalimide groups was accomplished in

refluxing ethanol and hydrazine to obtain the spacer bearing two free amines (Scheme 2, step c). Mono Boc protection yielded the mono-amine **104** in 37% yield (Scheme 2, step d).



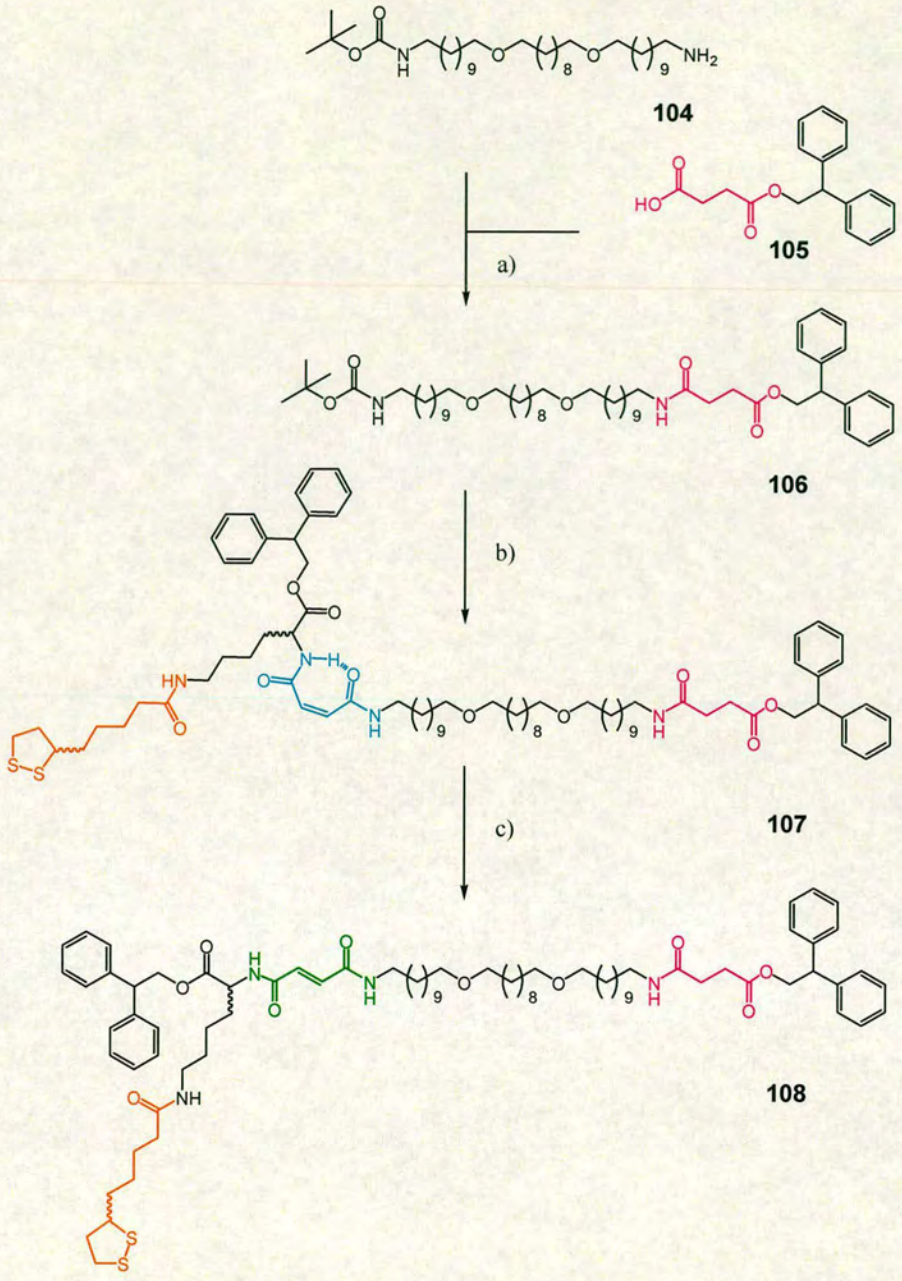
Scheme 2. Synthesis of the spacer. a) Tf_2O, pyridine, CH_2Cl_2, 81%; b) **101**, 1,10-decanediol, proton sponge, CH_2Cl_2/CH_3CN (5:1), reflux, 71%; c) N_2H_4, EtOH, reflux, 95%; d) $O(CO_2tBu)_2$, $CHCl_3$, 37%.

4.3.3 Synthesis of the thread.

Cis Thread **107** will be divided and discussed in three different parts as shown by scheme 3 which comprise:

- the spacer (**104**)
- the maleic station (**100**)
- the succinic station (**105**), which synthesis has already reported by Leigh and coworkers. [30]

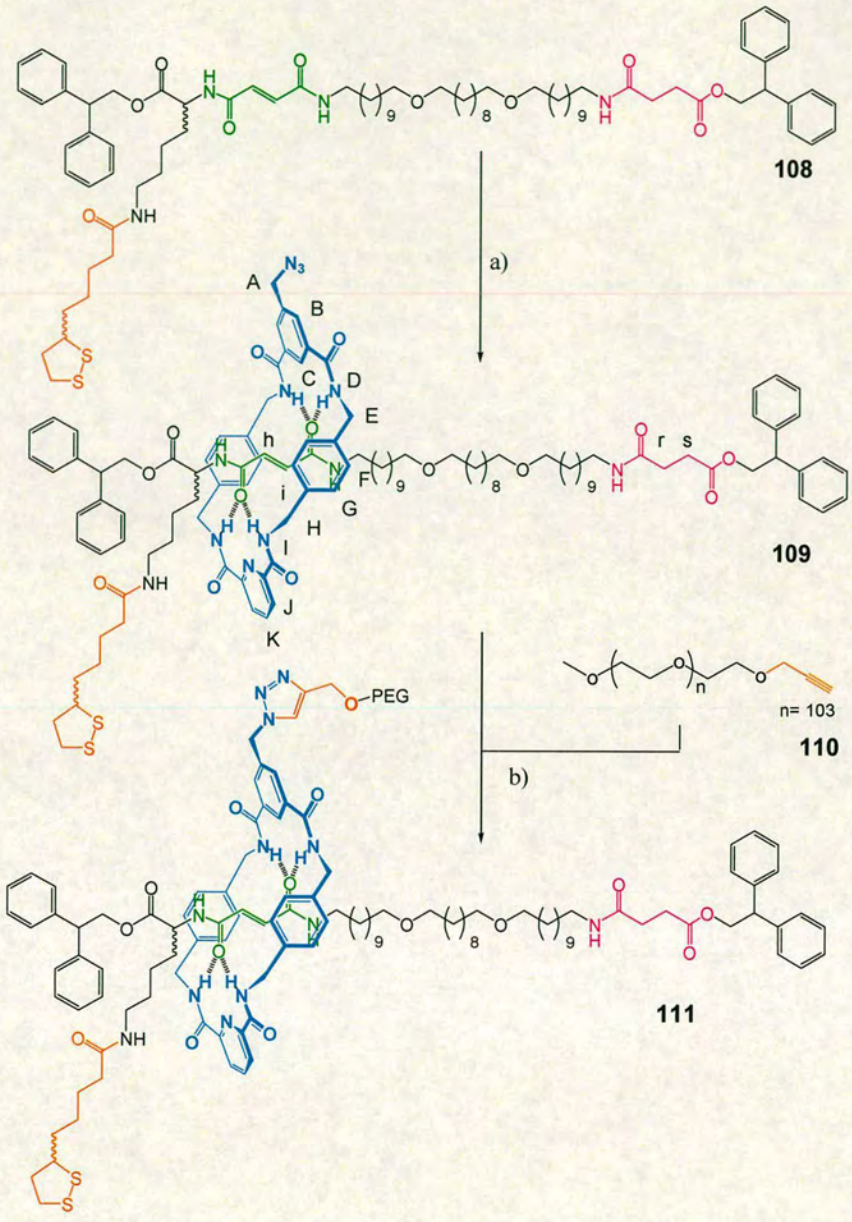
The succinic station **105** and the mono-protected spacer **104** (just described) were coupled together using EDCI and DMAP yielding fragment **106** in 91% yield (Scheme 3, step a). Removal of the Boc protecting group with TFA in CH_2Cl_2 produced the free amine necessary for further coupling with the maleic station described above (Scheme 1, compound **100**). *Cis* thread **107** was synthesized from coupling of **100** and deprotected **106** using EDCI and DMAP and isolated in a 61% yield (Scheme 3, step b). *Cis* thread **107** was isomerized to the *trans* isomer (thread **108**) with piperidine in CH_2Cl_2 (Scheme 3, step c).



Scheme 3. Synthesis of the *trans* thread **108**. Reagents and yields: a) EDCI.HCl, 4-DMAP, succinic station **105**, Et_3N, CH_2Cl_2, 91%; b) TFA/ CH_2Cl_2 (1:9), then **3** EDCI.HCl, 4-DMAP, Et_3N, CH_2Cl_2, 61%; c) piperidine, CH_2Cl_2, 2 d, 97%.

4.3.4 Synthesis of the rotaxane and attachment of the PEG linker.

Macrocyclization of the ¾-macrocycle fragment **43** bearing an endotopic nitrogen (from Chapter 2) and the corresponding dichloride compound **49A** obtained from 5-azidomethyl isophthalic acid **49** (from Chapter 2) around thread **108** in $CHCl_3$ yielded [2]rotaxane **109** in 41% yield (Scheme 4, step a). Attachment of the alkyne functionalized PEG linker **110** to the macrocycle (Scheme 4, step b) was achieved by exploiting the "click" reaction (chemistry that proved successful in the synthesis of functionalized small rotaxanes reported in chapter 2). Scheme 4 illustrates the synthesis of the final rotaxane and attachment of the PEG linker to obtain rotaxane-PEG **111**.



Scheme 4. Formation of rotaxane **109** and attachment of the PEG linker **110**. Reagents and yields: a) **43** and **49A** (from chapter 2), Et_3N, $CHCl_3$, 44%; b) $Cu(I)(CH_3CN)_4PF_6$, Functionalized PEG **110**, *t*BuOH, CH_2Cl_2, 89%.

4.3.4.1 Characterization: evaluation of rotaxane 109.

The ^1H NMR analysis of thread **108** and rotaxane **109** in $CDCl_3/CD_3OD$ (9:1) (400 MHz, 298K) indicates that the macrocycle predominantly resides over the fumaramide station, evidenced by significant shielding of the vinylic protons. The H_h

and H_i protons of the fumaramide group are strongly shielded in the rotaxane compared to the thread evidenced by a shift in their peak positions by 1.42 ppm, whereas the chemical shifts of the H_r and H_s protons of the succinic amide-ester group are only slightly shifted upfield by 0.13 ppm.

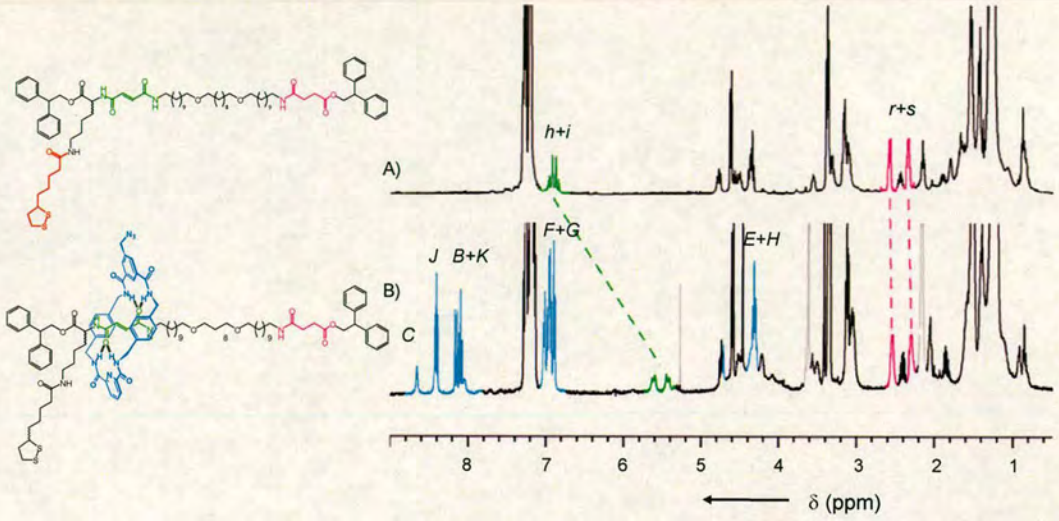


Figure 13. ^{1}H NMR [400 MHz, 298 K, $CHCl_3/CD_3OD$ (9:1)] spectra of thread **108** and rotaxane **110** (for hydrogen labels refer to scheme 4).

4.3.5 Preparation of the rotaxane-PEG monolayer on gold surface.

The gold surfaces (gold on mica) were cleaned by UV-ozone and subsequently dipped for 15 min in an ethanol buffer to reduce or minimize formation of a gold oxide layer. Samples were prepared by exposing a freshly cleaned gold surface to rotaxane-PEG **111** (0.1 mg/ml) and dodecyl sulphide (10-4 mol/l) in CH_2C1_2 solution for approx. 1 hour. After incubation the rotaxane-PEG modified surface was rinsed extensively with pure CH_2C1_2 to remove any non-adsorbed material. Alternatively, the preparation sequence may be modified with similar results in that one may dip the clean gold surface in the rotaxane-PEG (0.1 mg/ml) solution (for an hour), and subsequently dip it in a dodecyl sulfide (10-3 mol/l). The monolayer on gold surface was prepared at the Universitè Catholique de Louvain (Belgium) by Dr. Charles-Andrè Fustin.

4.3.5.1 Single Molecule Force Spectroscopy experiments on [2]rotaxane-PEG 111.

All the experiments have been performed using a multimode Veeco AFM (Veeco, Santa Barbara, CA) at the University of Liège (Belgium) under the supervision of Dr. Anne-Sophie Duwez. The samples were imaged in 'tapping mode' in air. After imaging, the AFM feedback was switched off and the instrument set for single molecule force spectroscopy on the gold adsorbed rotaxane-PEG layer. This latter experiment was performed in water to allow the PEG molecules to swell and attach to the AFM tip. Triangular cantilevers with a pyramidal tip (silicon nitride) and spring constant of 0.05 N/m were used for the pulling experiments. The loading rate of this device is 35 nN/m.

Figure 14 shows a typical AFM image of rotaxane-PEG molecules **111** adsorbed onto gold. The colour contrast in the AFM image indicates differences in height. In this image, the height difference from black to white represents a range of 5 nm. Flat Au terraces separated by valleys and channels can also be observed which is typical of a gold surface (i.e. big grains of Au) and is a result of the evaporation process. One is also able to see that the molecules are quite similar in size. Preliminary calculations taking into account the mean diameter of the observed structures (~ 8 nm) and the theoretical radius of a collapsed PEG chain ($N^{1/3}$= 5 nm, where N is the degree of polymerization) indicate that these structures are single polymer PEG chains. The chains adopted a globular conformation as a result of imaging in air (i.e. bad solvent conditions which cause the chains to collapse). One important and noteworthy feature of this image are the well separated rotaxane-PEG molecules, which ensures that during subsequent single molecule force spectroscopy experiments only one PEG chain at a time will be picked and stretched.

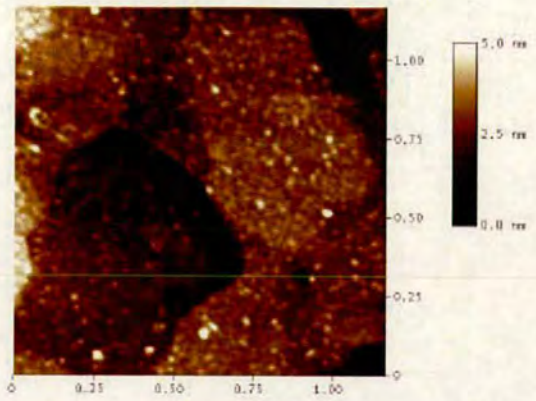


Figure 14: AFM image (1.25 × 1.25 μm^2) of rotaxane-PEG **111** grafted onto gold/mica surface. Colour contrast from black to white represents a range of 5 nm (indicated by the scale bar on the right of the image).

Figure 15 shows a typical force-distance curve of a bare tip and a rotaxane-PEG modified gold surface in water. In this experiment a continuous and repetitive approaching and retracting of the tip on the surface was performed until one PEG chain became attached to the tip. Once the PEG molecule was adsorbed on the tip the experiment proceeded as shown schematically in Figure 15. The first event (event 1) in Figure 15 corresponds to the tip-surface adhesion. At the end of event 1 the behaviour of the curve changes indicating the initiation of PEG stretching. From Figure 15, one may observe that a small plateau develops during PEG stretching which begins at a restoring force of around 70 pN. Taking into account that the necessary force for H-bond breakage is approximately 20 pN, then a force of 70pN may be attributed to the breaking of the 4 H-bonds which are expected to exist between the macrocycle and the fumaramide station. The width of the plateau is around 2.5 nm. This value is somewhat lower than anticipated, but within the expected range for the length of the rotaxane thread (4 nm). The difference may be due to fact that the piezo scanner used in these experiments was not close-loop, which may introduce relative high errors in z-displacement. Furthermore, since these experiments are preliminary studies of a rather complicated molecular system and the cantilevers have not been fine tuned and specifically calibrated, the measured distance is likely an underestimate. It is also important note that the long thread in these rotaxanes is expected to be in a somewhat collapsed state as a result of non-

ideal solvent conditions (water). Many possible scenarios may be envisioned that account for the short distance measured such as that the macrocycle does not travel the full length of the thread before reaching the succinic amide-ester station, or that the macrocycle is not directly over the fumaramide station to begin with, all of which may explain the smaller value for the distance measured by AFM data. Further work is required to understand this discrepancy, however, the distance of 2.5 nm is within the contour length of the thread. The event following the small plateau corresponds to the further stretching of the PEG until the chain detaches from the tip and the tip returns in its resting position (indicating by zero force on the curve of Figure 15).

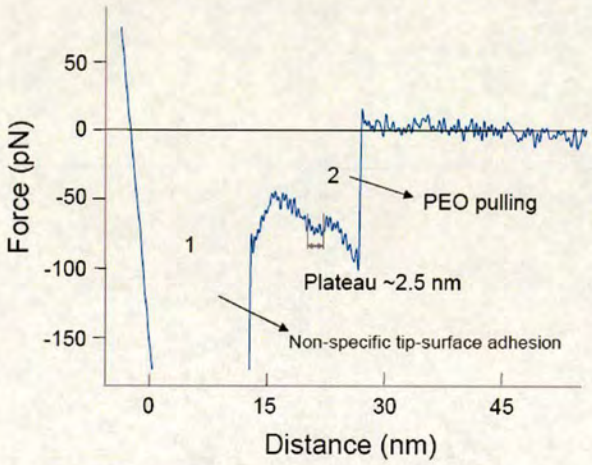


Figure 15. Typical force curve during the AFM pulling experiments in water on the rotaxane-PEG molecules **111** adsorbed on gold.

4.4 Conclusion.

In conclusion, preliminary results on the AFM experiments of rotaxane-PEG **111** attached to gold are promising, and data indicative of ring shuttling along the thread in these systems has been measured in the force curves. Nevertheless, further experiments are necessary and the experimental setup needs to be better tuned to accurately handle this complicated system in order to obtain more reliable and quantitative results. Pulling rate dependent experiments must also be performed to probe the energy landscape in different solvents and determine the kinetic off-rate constant of the interactions. To this end further experiments will be performed using

a PicoPlus instrument equipped with a closed loop piezo tip from Agilent Technologies which adapted and better suited for single molecule manipulation.

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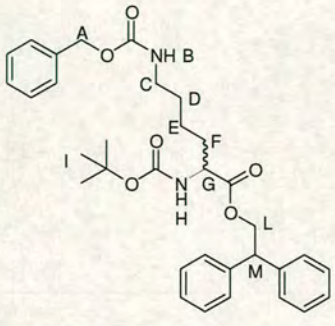
Experimental part

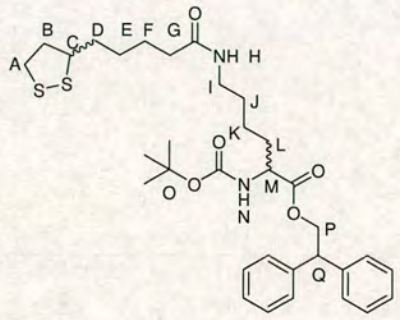
6-Benzyloxycarbonylamino-2-tert-butoxycarbonylamino-hexanoic acid 2,2-diphenyl-ethyl ester (98).



(D/L)-Boc-Lys(Cbz)-OH (960 mg, 2.52 mmol) was dissolved in CH_2Cl_2 (25 mL) and the solution was cooled to 0 °C. EDCI·HCl (773 mg, 4.03 mmol), 4-DMAP (31 mg, 0.25 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 30 min and then a solution of 2,2-Diphenyl-ethanol in dichloromethane (Sigma-Aldrich) (10 mL) (600 mg, 3.02 mmol) was added to the activated acid. The reaction mixture was stirred for 36 h, concentrated under reduced pressure and then CH_2Cl_2 was added (200 mL). The organic layer was washed with 1M HCl (50 mL), with $NaHCO_3$ (sat. aq., 50 mL), then further washed with brine (sat., 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (gradient elution: CH_2Cl_2 / MeOH 99.9:0.1 then CH_2Cl_2 / MeOH 98.5:1.5) to furnish compound **1** as a colourless oil (1.30 g, 92%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30-7.07 [m, 15H, Ar-H], 4.99 [s, 2H, CH_A], 4.89 [d, 1H, J = 7.8 Hz, NH_H], 4.70 [t, 1H, J = 9.6 Hz, CH_L], 4.60 [br t, 1H, NH_B], 4.46 [t, 1H, J = 9.6 Hz, $CH_{L'}$], 4.28 [t, 1H, J = 7.7 Hz, CH_G], 4.08 [dd, 1H, J = 5.6 Hz, J = 7.5 Hz, CH_M], 2.95 [q, 2H, J = 6.4 Hz, CH_C], 1.48-1.12 [m, 13H, CH_F, CH_D and CH_I], 1.11-0.84 [m, 2H, CH_E]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 172.5, 156.3, 155.3, 140.7, 140.5, 136.5, 128.7, 128.6,

128.2, 128.1, 126.9, 79.9, 67.3, 66.6, 53.4, 49.7, 40.5, 32.2, 29.2, 28.3, 22.0; FABMS: m/z = 561 $[M+H]^+$; HRMS : m/z = 561.2963 $[M+H]^+$ (anal. calcd. for $C_{33}H_{41}N_2O_6^+$: m/z = 561.2964).

2-tert-Butoxycarbonylamino-6-(5-[1,2]dithiolan-3-yl-pentanoylamino)-hexanoic acid 2,2-diphenyl-ethyl ester (99).

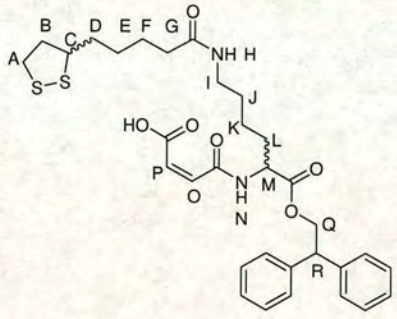


Pd/C (420 mg, 10%) was stirred under nitrogen for 5 min then a solution of **98** (1.18 g, 2.1 mmol) in THF (30 mL) was added. Nitrogen was replaced with hydrogen and the solution was stirred for 2 h until complete by TLC. Then the catalyser was filtered over a plug of celite and washed with THF. The solvent was removed under reduced pressure and the solid was dried until the complete removal of the solvent. The crude product was used directly for further reaction. Yield = 99%.

(D/L)-Lipoic acid (477 mg, 2.31 mmol) was dissolved in CH_2Cl_2 (15 mL) and the solution was cooled to 0 °C. EDCI·HCl (644 mg, 3.36 mmol), 4-DMAP (410 mg, 3.36 mmol) were added at 0 °C and the reaction mixture was allowed to stir at room temperature for 30 min. A solution of **98** (deprotected) from the previous hydrogenation reaction in CH_2Cl_2 (10 mL) was added to the activated acid. The reaction mixture was stirred for 18 h, concentrated under reduced pressure and then CH_2Cl_2 was added (200 mL). The organic layer was washed with 1M HCl (50 mL), with $NaHCO_3$ (sat. aq., 50 mL). The organic layer was further washed with brine (sat., 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash

chromatography (gradient elution: CH_2Cl_2 / MeOH 99:1 then CH_2Cl_2 / MeOH 97:3) to furnish compound **99** as a colourless oil (1.20 g, 93%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36-7.19 [m, 10H, Ar-H], 5.58 [br t, 1H, NH_H], 5.03 [d, 1H, J = 8.0 Hz, NH_N], 4.80 [t, 1H, J = 9.0 Hz, CH_P], 4.59 [t, 1H, J = 9.0 Hz, CH_P], 4.40 [t, 1H, J = 7.60 Hz, CH_M], 4.19 [br d, 1H, J = 5.2 Hz, CH_Q], 3.58 [q, 1H, J = 6.6 Hz, CH_C], 3.26-3.02 [m, 4H, CH_A and CH_I], 2.48 [m, 1H, CH_D], 2.17 [t, 2H, J = 7.4 Hz, CH_G], 1.92 [m, 1H, CH_D], 1.80-1.58 [m, 4H, CH_F and CH_L], 1.58-0.90 [m, 17H, CH_B, CH_E, CH_J, CH_K and CH_O]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 172.6, 157.5, 155.3, 140.7, 140.5, 128.6, 128.1, 126.9, 79.8, 67.2, 56.4, 53.0, 49.7, 40.2, 39.0, 38.4, 36.4, 34.6, 32.2, 28.9, 28.2, 25.6, 22.2; FABMS: m/z = 614 $[M]^+$; HRMS : m/z = 614.2848 $[M]^+$ (anal. calcd. for $C_{33}H_{46}N_2O_5S_2^+$: m/z = 614.2848).

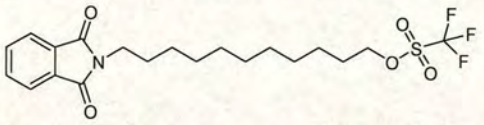
3-[1-(2,2-Diphenyl-ethoxycarbonyl)-5-(5-[1,2]dithiolan-3-yl-pentanoylamino)-pentylcarbamoyl]-acrylic acid (100).



A solution of **99** (1.0 g, 1.62 mmol) in CH_2Cl_2 / TFA 9:1 (10 mL) was stirred for 2h at 0°C and then the reaction was allowed to stir at rt for other 3 h. The reaction mixture was concentrated under reduced pressure to remove most of the TFA and then CH_2Cl_2 was added (200 mL). The organic layer was washed with $NaHCO_3$ (sat. aq., 20 mL) further washed with brine (sat., 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was used directly for further reaction. Yield = 99%.

A solution of the previous amine and Et_3N (0.25 ml, 1.62 mmol) in anhydrous THF, was cooled to 0°C, then maleic anhydride (164 mg, 1.68 mmol) in THF (10ml) was added dropwise under nitrogen atmosphere. The solution was allowed to warm up to room temperature and stirred overnight. THF was removed under reduced pressure and the crude material was purified by flash chromatography (gradient elution: CH_2Cl_2 / MeOH 98:02 and 0.1% HOAc then CH_2Cl_2 / MeOH 95:5 and 1% HAc) to furnish compound **100** as a colorless solid (892 mg, 90%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.54 [d, 1H, J = 4.0 Hz, NH_N], 7.27-7.16 [m, 10H, Ar-H], 6.58 [d, 1H, J = 12.8 Hz, CH_P], 6.32 [d, 1H, J = 12.8 Hz, CH_O], 6.17 [t, 1H, J = 5.9 Hz, NH_H], 4.81 [dd, 1H, J = 3.0 Hz, J = 7.8 Hz, CH_Q], 4.62 [dd, 1H, J = 3.0 Hz, J = 7.8 Hz, CH_Q], 4.41 [t, 1H, J = 7.7 Hz, CH_M], 4.32 [dd, 1H, J = 5.9 Hz, J = 6.1 Hz, CH_R], 3.56 [m, 1H, CH_C], 3.29-3.04 [m, 4H, CH_A and CH_I], 2.45 [m, 1H, CH_D], 2.22 [t, 2H, J = 7.36 Hz, CH_G], 1.91 [m, 1H, CH_D], 1.75-1.53 [m, 6H, CH_B, CH_J and CH_L], 1.51-0.95 [m, 6H, CH_F, CH_E and CH_K]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 174.3, 170.3, 166.4, 165.3, 140.6, 140.3, 135.9, 128.6, 128.1, 126.9, 67.5, 53.4, 53.2, 49.7, 40.2, 38.4, 37.5, 36.4, 35.5, 34.4, 29.1, 28.7, 25.4; FABMS: m/z = 612 $[M+H]^+$; HRMS : m/z = 613.2406 $[M+H]^+$ (anal. calcd. for $C_{32}H_{41}N_2O_6S_2^+$: m/z = 613.2406).

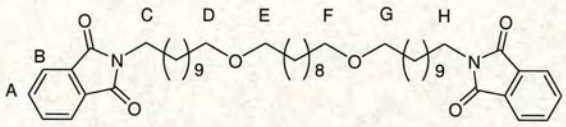
Preparation of Trifluoro-methanesulfonic acid 11-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-undecyl ester (101).



To an ice-cooled flask containing dried, anhydrous CH_2Cl_2 (60 mL) under nitrogen was added trifluoromethanesulfonic anhydride (10 g, 35.4 mmol), followed by anhydrous pyridine (2.9 mL, 35.4 mmol). Fuming was observed, and a white precipitate formed. The cooling bath was removed, and a solution of **76** (8.04 g, 25.3 mmol) in anhydrous

CH_2Cl_2 (40 mL) was added dropwise over a period of 15 min. The solution was stirred for 3 h at room temperature, and then water (100 mL) was added to quench the reaction. Then CH_2Cl_2 (100 mL) was added and the solution was washed twice with water (2 x 100 mL), brine (200 mL), dried over $MgSO_4$ and evaporated under reduced pressure to yield a orange oil. The oil was dissolved in CH_2Cl_2/Et_2O (1:1) (20 mL) and loaded onto a 3 cm bed of silica gel. The product was eluted with CH_2Cl_2 and diethyl ether, taking care not to coelute the more polar yellow byproducts. The solvents were removed under reduced pressure to yield **101** as a pale brown oil (9.22 g, 20.5 mmol, 81%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (dd, 2H, J = 5.4, 3.1 Hz, CH_b), 7.70 (dd, 2H, J = 5.4, 3.1 Hz, CH_a), 4.53 (t, 2H, J = 6.5 Hz, CH_f), 3.67 (br t, J = 7.3 Hz, 2H, CH_c), 1.81 (m, 2H, CH_e),1.66 (m, 2H, CH_d), 1.43-1.26 (m, 14H, alkyl chain); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168. 5, 133.8, 132.1, 123.1, 118.6 (q, J = 319.4 Hz, CF_3), 77.7, 38.0, 29.4, 29.3, 29.2, 29.1, 29.0, 28.8, 28.5, 26.8, 25.0; ^{19}F NMR (235 MHz, $CDCl_3$): δ = -75.85 (s, CF_3).

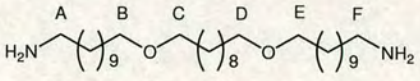
Preparation of 2-(11-{10-[11-(1,3-Oxo-isoindol-2-yl)-undecyloxy]-decyloxy}-undecyl)-isoindole-1,3-dione (102).



To a mixture of 1,10-decanediol (0.94 g, 5.41 mmol), compound **101** (6.9 g, 15.40 mmol) and 1,8-bis(dimethylamino)naphthalene (proton sponge, 8.3 g, 38.7 mmol) under nitrogen atmosphere was added CH_2Cl_2 (25 mL) and CH_3CN (5 mL). The yellow solution was refluxed under nitrogen for 78 h. During this time, the reaction had turned dark orange/brown, and a precipitate had formed. The precipitate was filtered and CH_2Cl_2 was added to the organic phase and then washed with 1M HCl, H_2O, dried over anhydrous $MgSO_4$, and evaporated to dryness. The crude material was purified by flash

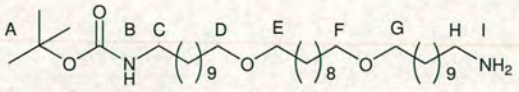
chromatography (gradient elution: CH_2Cl_2 / EtOAc 99:1 then CH_2Cl_2 / EtOAc 98:2) to furnish compound **102** as a white solid (3.00 g, 71%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 [dd, 4H, J = 5.1 Hz, J = 3.0 Hz, Ar-H_B], 7.70 [dd, 4H, J = 5.1 Hz, J = 3.0 Hz, Ar-H_A], 3.66 [t, 4H, J = 7.3 Hz, CH_C and CH_H], 3.37 [t, 8H, J = 6.7 Hz, CH_{DEFG}], 1.71-1.48 [m, 12H, N-CH_2-$\underline{CH_2}$ and O-CH_2-$\underline{CH_2}$], 1.37-1.18 [m, 40H, CH_2 Alkyl]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 172.2, 133.5, 131.8, 122.8, 70.7, 37.9, 29.4, 29.3, 29.2, 29.1, 28.9, 28.3, 26.6, 25.9; FABMS: m/z = 773 $[M+H]^+$; HRMS : m/z = 773.5468 $[M+H]^+$ (anal. calcd. for $C_{48}H_{73}N_2O_6^+$: m/z = 773.5468).

Peparation of 11-[10-(11-Amino-undecyloxy)-decyloxy]-undecylamine (103).



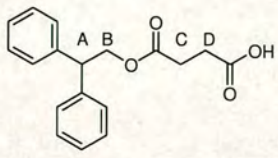
A solution of **102** (3.0 g, 3.88 mmol) and N_2H_4 (1.51 mL, 31.0 mmol) in absolute ethanol was refluxed for 4 h. The voluminous white precipitate was separated by filtration (2x), and then the filtrate was concentrated in vacuo to an off-white solid. The white solid was triturated twice with $CHCl_3$ (200 mL) and filtered. The filtrate was then dried over anhydrous Na_2SO_4. After filtration of the inorganic salt the filtrate was concentrated in vacuo to yield a glassy white solid (1.90 g, 95%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.37 [t, 8H, J = 6.7 Hz, CH_{BCDE}], 2.66 [t, 4H, J = 7.0 Hz, CH_A and CH_F], 1.70 [br s, 4H, NH_2], 1.54 [m, 8H, O-CH_2-$\underline{CH_2}$], 1.42 [m, 4H, N-CH_2-$\underline{CH_2}$], 1.35-1.17 [m, 40H, CH_2 Alkyl]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 42.2, 33.9, 29.8, 29.6, 29.56, 29.52, 29.5, 26.8, 26.2; FABMS: m/z = 512 $[M]^+$; HRMS : m/z = 512. 3477 $[M]^+$ (anal. calcd. for $C_{32}H_{68}N_2O_2^+$: m/z = 512.5280).

Preparation of {11-[10-(11-Amino-undecyloxy)-decyloxy]-undecyl}-carbamic acid tert-butyl ester (104).



To a solution of **103** (1.8 g, 3,51 mmol) in anhydrous $CHCl_3$ (50 mL) was added a solution of Boc_2O (383 mg, 1.75 mmol) in $CHCl_3$ (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 4 h. $CHCl_3$ was removed under reduced pressure and the crude material was purified by flash chromatography [eluant: CH_2Cl_2 / MeOH / NH_3(aq) 94:5:1] to furnish compound **104** as a colourless solid (770 mg, 37%); mp: 115-119 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.59 [br s, 1H, NH_B], 3.37 [t, 8H, J = 6.7 Hz, CH_{DEFG}], 3.09 [m, 2H, CH_C], 2.67 [t, 2H, J = 7.0 Hz, CH_H], 1.63 [br s, 2H, NH_2], 1.54 [m, 8H, O-CH_2-$\underline{CH_2}$], 1.46-1.37 [m, 12H, N-CH_2-$\underline{CH_2}$ and CH_A], 1.36-1.20 [m, 40H, CH_2 Alkyl]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 156.0, 71.0, 42.1, 40.6, 33.6, 30.0, 29.7, 29.65, 29.60, 29.55, 29.4, 29.3, 28.4, 26.9, 26.2, 22.1; FABMS: *m/z* = 613 $[M+H]^+$; HRMS : *m/z* = 613.5889 $[M+H]^+$ (anal. calcd. for $C_{37}H_{77}N_2O_4^+$: *m/z* = 613.5883).

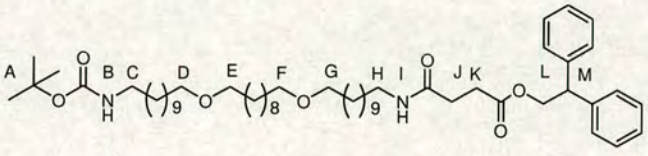
Preparation of 2,2-Diphenylethyl Succinic acid mono ester (105).



To a stirred solution of 2,2-diphenylethanol (3.00 g, 15.0 mmol) in CH_2Cl_2 (150 mL) was added one drop of Et_3N and a solution of succinic anhydride (1.66 g, 16.7 mmol) in CH_2Cl_2 (25 mL) added slowly over 30 mins. After 16 h the solution was reduced in volume and recrystallized from CH_2Cl_2 (10 mL) to obtain a colourless solid (4.00 g,

90%). mp = 103-104 °C; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 7.38-7.21 [m, 10H, Ar-H], 4.61 [d, 2H, *J* = 7.7 Hz, CH_B], 4.35 [t, 1H, *J* = 7.7 Hz, CH_A], 2.40 [m, 4H, CH_C and CH_D]; ^{13}C NMR (400 MHz, d_6-DMSO): δ = 173.9, 172.4, 141.8, 128.8, 128.3, 127.0, 66.5, 49.6, 29.1, 28.9; FABMS: *m/z* = 299 $[M+H]^+$. Compound **105** was prepared as reported by Leigh and coworkers and showed spectroscopic data according to literature. [1]

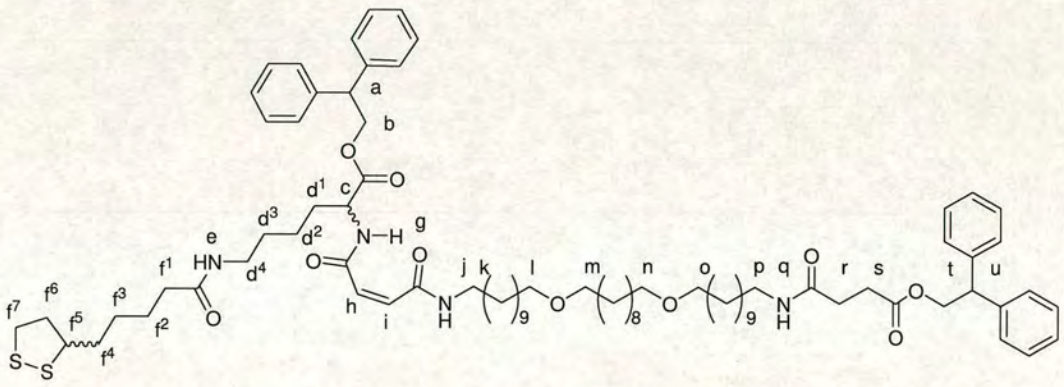
Preparation of N-{11-[10-(11-tert-Butoxycarbonylamino-undecyloxy)-decyloxy]-undecyl}-succinamic acid 2,2-diphenyl-ethyl ester (106).



Compound **105** (406 mg, 1.36 mmol) was dissolved in CH_2Cl_2 (15 mL) and the solution was cooled to 0 °C. EDCI·HCl (383 mg, 2.0 mmol), 4-DMAP (244 mg, 2.0 mmol) were added at 0 °C and the reaction mixture was allowed to stir at room temperature for 30 min. A solution of **104** (10 mL) (760 mg, 1.24 mmol) was added to the activated acid. The reaction mixture was stirred for 24 h, concentrated under reduced pressure and then CH_2Cl_2 was added (200 mL). The organic layer was washed with hydrochloric acid (1 N, 50 mL), with $NaHCO_3$ (sat. aq., 50 mL). The organic layer was further washed with brine (sat., 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluant: CH_2Cl_2 / MeOH 98:2) to furnish compound **106** as a white solid (1.01 g, 91%); mp: 86-88 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33-7.17 [m, 10H, Ar-H], 5.52 [br t, 1H, NH_I], 4.62 [d, 2H, *J* = 7.6 Hz, CH_L], 4.51 [br t, 1H, NH_B], 4.34 [t, 1H, *J* = 7.6 Hz, CH_M], 3.37 [t, 8H, *J* = 6.7 Hz, CH_{DEFG}], 3.17 [q, 2H, *J* = 6.8 Hz, CH_H], 3.09 [q, 2H, *J* = 6.4 Hz, CH_C], 2.32 [t, 2H, *J* = 6.8 Hz, CH_K], 2.32 [t, 2H, *J* = 6.9 Hz, CH_J], 1.61-1.49 [m, 8H, O-CH_2-$\underline{CH_2}$], 1.48-1.37

[m, 13H, N-CH_2-$\underline{CH_2}$ and CH_A], 1.36-1.18 [m, 40H, CH_2 Alkyl]; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 172.9, 172.8, 171.1, 141.0, 128.5, 128.1, 126.8, 70.9, 66.8, 49.7, 40.6, 39.6, 31.0, 29.7, 29.6, 29.54, 29.50, 29.46, 29.3, 28.4, 26.8, 26.1; FABMS: *m/z* = 893 $[M+H]^+$; HRMS : *m/z* = 893.6986 $[M+H]^+$ (anal. calcd. for $C_{55}H_{93}N_2O_7^+$: *m/z* = 893.6982).

Preparation of (Z)-2-{3-[11-(10-{11-[3-(2,2-Diphenyl-ethoxycarbonyl)-propionylamino]-undecyloxy}-decyloxy)-undecylcarbamoyl]-acryloylamino}-6-(5-[1,2]dithiolan-3-yl-pentanoylamino)-hexanoic acid 2,2-diphenyl-ethyl ester (107).

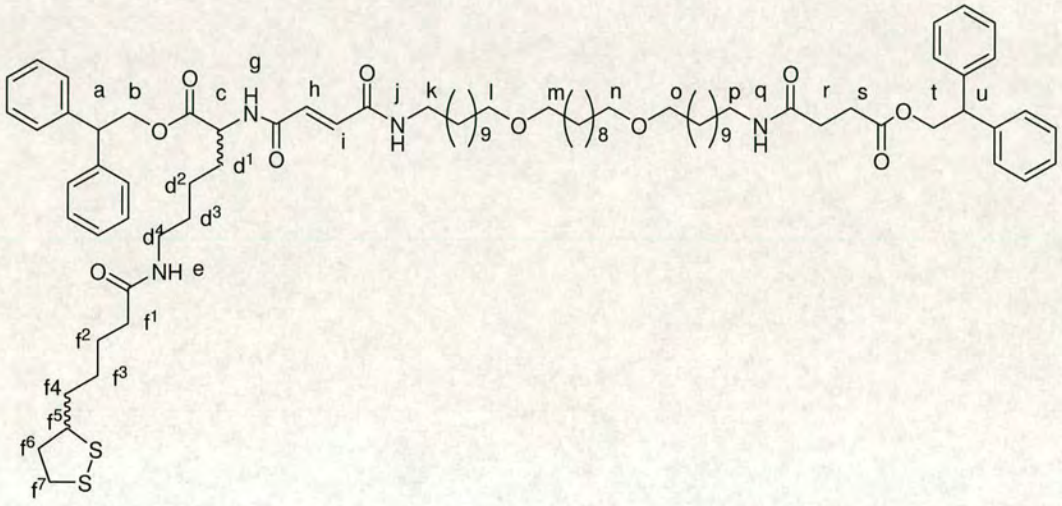


A solution of **106** (1.0 g, 1.12 mmol) in CH_2Cl_2 / TFA 9:1 (40 mL) was stirred for 3 h. The reaction mixture was concentrated under reduced pressure to remove TFA and then CH_2Cl_2 (300 mL) was added. The organic layer was washed with $NaHCO_3$ (sat. aq., 50 mL) dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was triturated twice with diethyl ether to give the product as colourless white solid (875 mg, 98%).

Carboxylic acid **100** (753 mg, 1.23 mmol) was dissolved in CH_2Cl_2 (25 mL) and the solution was cooled to 0 °C. EDCI·HCl (344 mg, 1.8 mmol), 4-DMAP (220 mg, 1.8 mmol) and Et_3N (305 mg, 3.02 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 30 min. A solution of amine in CH_2Cl_2 (15 mL)

(875 mg, 1.12 mmol) was added to the activated acid. The reaction mixture was stirred for 24 h, concentrated under reduced pressure and then dichloromethane was added (300 mL). The organic layer was washed once with 1M HCl (50 mL) once with $NaHCO_3$ (sat. aq., 50 mL) then concentrated under reduced pressure. The crude material was purified by flash chromatography [eluant: CH_2Cl_2 / MeOH 96:4] to furnish compound **107** as a colourless oil (947 mg, 61%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ = 7.29-7.10 [m, 20H, Ar-H], 6.25 [s, 4H, CH_h and CH_i], 4.73 [dd, 1H, J = 2.8 Hz, J = 8.2 Hz, CH_b], 4.60-4.49 [m, 3H, CH_t and CH_b], 4.38-4.24 [m, 3H, CH_a, CH_c and CH_u], 3.51 [m, 1H, CH-f^5], 3.35 [t, 8H, J = 6.8 Hz, CH_{lmno}], 3.23 [t, 2H, J = 7.2 Hz, CH_p], 3.17-2.99 [m, 6H, CH_k, CH-f^7, CH-d^4], 2.51 [t, 2H, J = 7.0 Hz, CH_s], 2.39 [m, 1H, CH-f^6], 2.27 [t, 2H, J = 7.0 Hz, CH_r], 2.17-2.04 [m, 8H, CH-f^4, CH-f^1, CH-d^1, CH-d^3], 1.85 [m, 1H, CH-f^6], 1.67-1.43 [m, 14H, CH-f^2, CH, O-CH_2-$\underline{CH_2}$ and N-CH_2-$\underline{CH_2}$], 1.43-1.22 [m, 44H, CH-f^3, CH-d^2 and alkyl CH_2]. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.0, 172.6, 171.0, 157.4, 140.8, 128.4, 128.3, 128.0, 127.9, 126.7, 126.6, 70.7, 67.1, 66.6, 56.2, 49.5, 40.1, 39.4, 38.2, 36.2, 34.4, 30.8, 29.5, 29.4, 29.37, 29.33, 29.30, 29.2, 29.1, 29.0, 28.7, 26.8, 26.7, 26.0. FABMS: m/z = 1387 $[M+H]^+$; HRMS : m/z = 1387.8671 $[M+H]^+$ (anal. calcd. for $C_{82}H_{123}N_4O_{10}S_2^+$: m/z = 1387.8680).

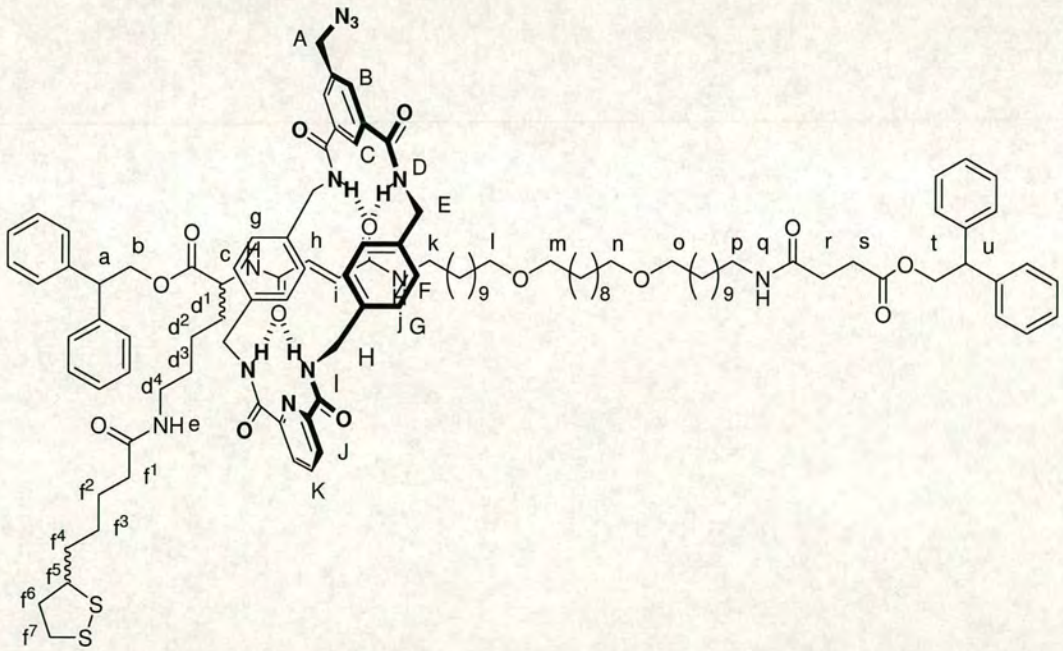
Preparation of (E)-2-{3-[11-(10-{11-[3-(2,2-Diphenyl-ethoxycarbonyl)-propionylamino]-undecyloxy}-decyloxy)-undecylcarbamoyl]-acryloylamino}-6-(5-[1,2]dithiolan-3-yl-pentanoylamino)-hexanoic acid 2,2-diphenyl-ethyl ester (108).



A solution of *cis* Thread **107** (500 mg, 0.36 mmol) piperidine (0.15 mg, 0.0018 mmol) in CH_2Cl_2 (25 ml) was stirred for 48h. Then CH_2Cl_2 was added (100 ml) and the organic layer was washed only once with 1M HCl (50 mL). The organic layer was concentrated under reduced pressure to furnish compound **108** as yellow solid (485 mg, 97%). mp: 190-194 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34-7.10 [m, 20H, Ar-H], 6.98 [d, 1H, *J* = 15.0 Hz, CH_h or CH_i], 6.90 [d, 1H, *J* = 15.0 Hz, CH_h or CH_i], 6.67 [br d, 1H, NH_g], 5.87 [br t, 1H, NH_J], 5.71 [br t, 1H, NH_q], 4.75 [dd, 1H, *J* = 2.8 Hz, *J* = 8.1 Hz, CH_b], 4.61 [d, 2H, *J* = 7.6 Hz, CH_t], 4.59-4.44 [m, 1H, CH_c], 4.42-4.27 [m, 3H, CH_a, CH_b and CH_u], 3.54 [m, 1H, CH-f^5], 3.38 [t, 8H, *J* = 6.7 Hz, CH_{lmno}], 3.21-3.00 [m, 8H, CH_p, CH_k, CH-f^7, CH-d^4], 2.57 [t, 2H, *J* = 6.8 Hz, CH_s], 2.39 [m, 1H, CH-f^4], 2.27 [t, 2H, *J* = 6.8 Hz, CH_r], 2.18-2.01 [m, 8H, CH-f^6, CH-f^1, CH-d^1, CH-d^3], 1.89 [m, 2H, CH-f^4],

1.75-1.00 [m, 58H, CH-f^2, O-CH_2-$\underline{CH_2}$, N-CH_2-$\underline{CH_2}$, CH-f^3, CH-d^2 and alkyl CH_2]; ^{13}C NMR (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ = 173.1, 172.0, 140.9, 128.5, 128.4, 128.1, 128.0, 126.7, 126.6, 70.9, 67.2, 66.9, 56.2, 49.6, 39.7, 38.4, 31.7, 31.1, 30.4, 29.5, 29.4, 29.37, 29.30, 29.2, 29.1, 29.0, 28.7, 26.8, 26.7, 22.5; FABMS: *m/z* = 1387 $[M+H]^+$; HRMS : *m/z* = 1387.8682 $[M+H]^+$ (anal. calcd. for $C_{82}H_{123}N_4O_{10}S_2^+$: *m/z* = 1387.8680).

Preparation of [2](1,9,16,19,22-Pentaaza-2,8,17,21-tetraaoxo-5-(azidomethyl)-3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-((E) -2-{3-[11-(10-{11-[3-(2,2-Diphenyl-ethoxycarbonyl)-propionylamino]-undecyloxy}-decyloxy)-undecylcarbamoyl]-acryloylamino}-6-(5-[1,2]dithiolan-3-yl-pentanoylamino)-hexanoic acid 2,2-diphenyl-ethyl ester-rotaxane (109).

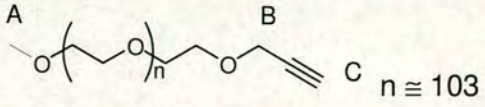


A stirred solution of 5-Azidomethyl-isophthalic acid **50** (500 mg, 2.26 mmol), $SOCl_2$ (10ml) and $(COCl)_2$ (0.5 mL) was heated under reflux for 12 hours. The solvent was removed under reduced pressure and the sample was kept overnight under vacuum to

remove thionyl chloride. Then, CH_2Cl_2 was added and the solvent was removed in vacuum. This step has been repeated for 4 times. The crude product was used directly for further reaction.

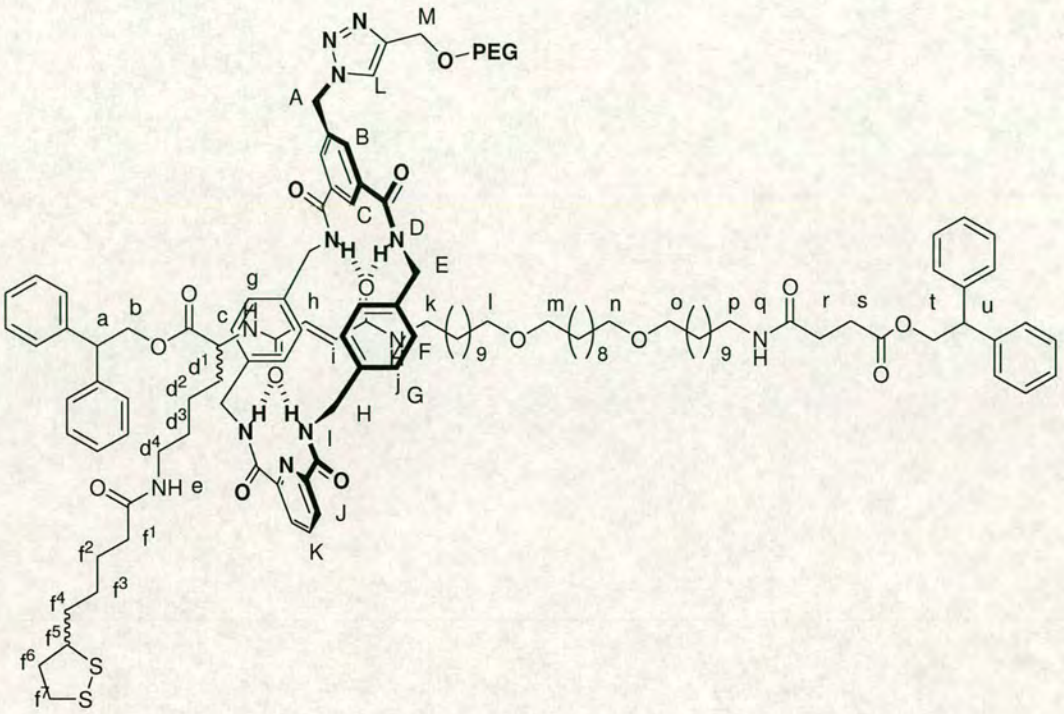
Thread **108** (670 mg, 0.483 mmol) compound **44** (1.95 g, 4.83 mmol) and Et_3N (1.35 mL, 9.66 mmol) in anhydrous $CHCl_3$ (50mL) were stirred vigorously whilst solution of 5-Azidomethyl-isophthaloyl dichloride (1.07 g, 4.83 mmol) in anhydrous $CHCl_3$ (20 ml) was simultaneously added over a period of 2 h using motor-driven syringe pumps. After 24 h the resulting suspension was filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography [eluant: CH_2Cl_2 / EtOAc / MeOH 20:78:2] to furnish compound **109** as a colourless oil (419 mg, 44%); ^1H NMR (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ = 8.55 [s, 1H, Ar-H_C], 8.30 [t, 2H, J = 6.9 Hz, Ar-H_J], 8.06 [s, 2H, Ar-H_B], 7.99 [t, 1H, J = 7.9 Hz, Ar-H_K], 7.23-6.98 [m, 20H, Ar-H_{thread}], 6.97-6.72 [m, 8H, Ar-H_F and CH_G], 5.51 [d, 1H, J = 14.6 Hz, CH_h or CH_i], 5.32 [d, 1H, 14.6 Hz, CH_h or CH_i], 4.63 [m, 3H, CH_A and CH_b], 4.50 [s, 2H, CH_t], 4.48 [s, 1H, CH_b] 4.45-4.07 [m, 11 H, CH_c, CH_a, CH_u, and CH_E and CH_H], 3.51 [m, 1H, CH-f^5], 3.25 [m, 8H, CH_{lmno}], 3.08-2.78 [m, 8H, CH_p, CH_k, CH-f^7, CH-d^4], 2.44 [t, 2h, J = 6.6 Hz, CH_s], 2.31 [m, 1H, CH-f^4], 2.20 [t, 2H, J = 6.2 Hz, CH_r], 2.11-1.90 [m, 8H, CH-f^6, CH-f^1, CH-d^1, CH-d^3], 1.75 [m, 1H, CH-f^4], 1.57-0.90 [m, 58H, CH-f^2, CH, O-CH_2-$\underline{CH_2}$, N-CH_2-$\underline{CH_2}$, CH-f^3, CH-d^2 and alkyl CH_2]; ^{13}C NMR (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ = 173.1, 171.4, 165.8, 164.0, 149.9, 140.1, 129.4, 129.3, 129.1, 128.9, 128.5, 128.2, 128.1, 128.0, 127.9, 126.8, 126.7, 126.6, 125.1, 125.0, 71.0, 66.9, 66.8, 56.4, 54.1, 40.2, 39.5, 38.4, 29.6, 29.5, 29.40, 29.36, 29.21, 29.30, 29.06, 26.8, 26.1; FABMS: m/z = 1977 $[M]^+$; HRMS : m/z = 1977.0941 $[M]^+$ (anal. calcd. for $C_{114}H_{152}N_{12}O_{14}S_2^+$: m/z = 1977.0992).

Functionalization of PEG: n-(2-Methoxy-ethoxy)-n-ethoxy–propyne (110).



PEG (4.60 g, 1.00 mmol; Mw: 4600) was dried by several azeotrope cycles with toluene and then dissolved in dry THF (100 mL) under Ar. NaH (28 mg, 1.2 mmol; 60% in paraffin oil) was suspended in dry THF (20 mL) under Ar. The solution containing the polymer was slowly added to the NaH suspension at 0 ^{0}C and the mixture was let to stir at RT for 90 min. A solution of propargyl bromide (160 mg, 1.2 mmol, solution in toluene) in dry THF (20 mL) was then added at 0°C. The reaction mixture was stirred for 24 h at RT. A saturated solution of NH_4Cl aq (50 mL) was added to the reaction medium and the mixture was extracted with CH_2Cl_2 (300 mL). The organic layer was washed with water (30 mL), dried over Na_2SO_4 and then CH_2Cl_2 was removed under vacuum. The alkyne-functionalized PEG was finally precipitated in cold diethyl ether from THF and dried 24 h under vacuum (yield: 88%); ^{1}H NMR ($CDCl_3$): 3.85-3.47 (m, 418H, PEG backbone), 3.38 (s, 3H, CH_A), 4.19 (d, 2H, CH_B), 2.44 (t, 1H, CH_C).

Preparation of [2](1,9,16,19,22-Pentaaza-2,8,17,21-tetraaoxo-5-{4-[n-(2-Methoxy-ethoxy)-n-(ethoxy)-ethoxymethyl]-[1,2,3]triazol-1-ylmethyl}-3,7,11,14,18,20,24,27-tetrabenzocyclohexacosane))-((E) -2-{3-[11-(10-{11-[3-(2,2-Diphenyl-ethoxycarbonyl)-propionylamino]-undecyloxy}-decyloxy)-undecylcarbamoyl]-acryloylamino}-6-(5-[1,2]dithiolan-3-yl-pentanoylamino)-hexanoic acid 2,2-diphenyl-ethyl ester-rotaxane (111).



Rotaxane **109** (135 mg, 0.068 mmol), Tetrakis-$(CH_3CN)_4$-Copper(I)-hexafluorophosphate (38 mg, 0.102 mmol) Alkyne-functionalized PEG **110** (Mw = 4600; 104 mg, 0.046 mmol) in CH_2Cl_2 (1 mL) and *tert*-butanol (1 mL) were stirred vigorously. The reaction mixture was stirred for 18 h until complete by TLC then concentrated under reduced pressure. CH_2Cl_2 was added (40 mL) and the organic phase was washed with EDTA 1M (aq) (5 ml) to remove the copper. The organic phase was separated and CH_2Cl_2 (20 ml) was added to the aqueous solution to recover the product. The two organic fractions were combined and washed with 1 mL of H_2O. To the latter aqueous fraction CH_2Cl_2 was added (25 mL) to recover the product. The organic fractions were combined and the solvent was removed under reduced pressure. The sample was triturated twice with toluene and twice with CH_2Cl_2. The crude material was left overnight under vacuum (267 mg, 89%). The product (267 mg, 0.041 mmol) was dissolved in the minimum amount of CH_2Cl_2 and added slowly to a stirred solution of hexane (200 mL). Yellow particles were filtered through a Millipore system (Cellulose nitrate; porosity = 0.45 μm). The solid was left under vacuum for 18 h to furnish a

yellow-orange solid (259 mg, 98%). (400 MHz, $CDCl_3/CD_3OD$ 9:1): δ = 8.50 [s, 1H, Ar-H_C], 8.22 [t, 2H, J = 6.9 Hz, Ar-H_J], 8.09-8.02 [m, 3H, Ar-H_B and CH_L], 7.89 [t, 1H, J = 7.9 Hz, Ar-H_K], 7.23-6.98 [m, 20H, Ar-H_{thread}], 6.97-6.72 [m, 8H, Ar-H_F and CH_G], 5.55-5.50 [m, 4H, CH_h, CH_i and CH_A], 4.63 [m, 3H, CH_A and CH_b], 4.50 [s, 2H, CH_t], 4.48 [s, 1H, CH_b] 4.45-4.07 [m, 11 H, CH_c, CH_a, CH_u, and CH_E and CH_H], 3.90-3.50 [m, 420H, PEG backbone and CH_M], 3.51 [m, 1H, CH-f^5], 3.25 [m, 8H, CH_{lmno}], 3.08-2.78 [m, 8H, CH_p, CH_k, CH-f^7, CH-d^4], 2.44 [t, 2h, J = 6.6 Hz, CH_s], 2.31 [m, 1H, CH-f^4], 2.20 [t, 2H, J = 6.2 Hz, CH_r], 2.11-1.90 [m, 8H, CH-f^6, CH-f^1, CH-d^1, CH-d^3], 1.75 [m, 1H, CH-f^4], 1.57-0.90 [m, 58H, CH-f^2, CH, O-CH_2-$\underline{CH_2}$, N-CH_2-$\underline{CH_2}$, CH-f^3, CH-d^2 and alkyl CH_2].

Preparation of the SAM

Gold surfaces (gold on mica) were cleaned by UV-ozone and end dipped for 15 min in EtOH to reduce the gold oxide. The clean gold surface was dipped for 1 hour in rotaxane-PEG (0.1 mg/mL) and dodecyl sulfide (10-4 mol/L) solution of CH_2Cl_2. The same result was obtained by first dipping the gold surface for 1 hour in the rotaxane-PEG (0.1 mg/mL) solution and subsequently dipping it in a dodecyl sulfide (10-3 mol/l) solution. Upon removal from the solution the surfaces were copiously rinsed with CH_2Cl_2. The concentrations and dipping time were optimised to have isolated rotaxanes on the surface.

References.

[1] A. Altieri, G. Bottari, F. Dehez, D. A. Leigh, J. K. Y. Wong, F. Zerbetto, *Angew. Chem., Int. Ed.* **2003**, *42*, 2296-2300.